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HEIKKI SARIN

BODY COMPOSITION, EXERCISE TRAINING, AND ENERGY AVAILABILITY AS DETERMINANTS OF IMMUNE SYSTEM AND CARDIOMETABOLIC SIGNATURES

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BODY COMPOSITION, EXERCISE TRAINING, AND ENERGY AVAILABILITY AS DETERMINANTS OF IMMUNE SYSTEM AND CARDIOMETABOLIC SIGNATURES

Heikki Vihtori Sarin

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Supervised by

Research professor Markus Perola

Department of Public Health Solutions, Genomics and Biobank Unit,

Finnish Institute for Health and Welfare,

Research Program for Clinical and Molecular Metabolism,

University of Helsinki,

Helsinki, Finland

Adjunct professor Kati Kristiansson

Department of Public Health Solutions, Genomics and Biobank Unit,

Finnish Institute for Health and Welfare,

Research Program for Clinical and Molecular Metabolism,

University of Helsinki,

Helsinki, Finland

Associate professor Juha Hulmi

Faculty of Sport and Health Sciences,

NeuroMuscular Research Center,

University of Jyväskylä,

Jyväskylä, Finland

Department of Physiology, Faculty of Medicine,

University of Helsinki,

Helsinki, Finland

Reviewed by

Assistant professor Katja Pahkala

Research Centre of Applied and Preventive Cardiovascular Medicine,

Paavo Nurmi Center,

Unit for Health and Physical Activity,

Centre for Population Health Research,

University of Turku,

Turku, Finland

Professor Harald HH Göring

Bennett Dyke and Jean MacCluer Endowed Professor,

Dept. of Human Genetics and South Texas Diabetes and Obesity Institute,

University of Texas Rio Grande Valley School of Medicine,

University of Texas Rio Grande Valley,

San Antonio, Texas, United States of America

Opponent

Professor Olli J. Heinonen

Paavo Nurmi Center,

Unit for Health and Physical Activity,

University of Turku,

Turku, Finland

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“The greater danger for most of us lies not in setting our aim too high and falling short; but in setting our aim too low, and achieving our mark.”

-Michelangelo

ABSTRACT

Many people engage in intense diet and exercise training programs in an attempt to pursue improved aesthetic appearance and health. Depending on the nature, dose, and intensity of these regimens, there may be positive or even negative effects on human physiology. Systems biology approaches can be used to evaluate human physiology and molecular signatures at the cellular level in a systematic manner. To date, the systems biology effects of body composition, exercise training, and energy availability alterations among healthy normal-weight individuals are relatively unknown.

In this thesis, the aforementioned gaps in knowledge were addressed by studying how i) substantial weight loss followed by weight regain, and ii) resistance training and associated body composition changes, affect the systems biology factors in healthy normal-weight individuals. The modulation of systems biology was investigated in longitudinal study settings by examining multi-dimensional datasets on serum metabolomics, plasma lipid metabolism regulating enzyme activities and protein contents, leukocyte transcriptomics, blood cell distribution, cytokine profile, and immunoglobulin G glycosylation.

In a sample of female physique athletes (n=42), a 20-week intense weight-loss period leading to substantial visceral fat mass reduction through high levels of exercise training and energy restriction resulted in a further improved cardiometabolic profile with beneficial modulation of serum lipid levels, high-density lipoprotein (HDL) profile, and inflammation-related biomarkers. Even preceding the weight-loss period, lipid profiles, inflammation markers, and other health-related biomarkers were at a more favourable level in the physique athletes compared to matched general-population females (n=58). In the replication cohort of age- and body mass index (BMI)-matched individuals, weight loss altered the cardiometabolic profile in a similar advantageous manner during a 7-year follow-up. Furthermore, the intense weight-loss period in the physique athletes affected multiple levels of the immune system and blood cell

proliferation. Dysregulated haematopoiesis and altered immune cell proliferation were indicated by the measured immune-system signatures. No long-term effects on systems biology factors were detected, as the majority of the observed changes on cardiometabolic profile, immune system, and blood-cell proliferation were reverted back during a subsequent voluntary 20-week weight-regain period.

In sample of healthy normal-weight men (n=59), a 16-week resistance training period led to increased levels of lean mass and reduced overall adiposity independent of weight change. The beneficial modulation of body composition was accompanied by an anti-atherogenic modulation of the serum metabolome as indicated by reduced levels of non-HDL cholesterol and apolipoprotein B. It was also shown that individuals with the poorest baseline body composition and biomarker profile benefitted the most from resistance training in terms of positive cardiometabolic signatures.

In conclusion, the results of this thesis show that further beneficial modulation of body composition even among healthy normal-weight individuals by exercise training and/or energy restriction has positive effects on the cardiometabolic profile, thus suggesting attenuated risk for future cardiovascular events. In addition, insight into novel molecular pathways mediating immune-system dysregulation and immunosuppression after prolonged periods of low-energy availability and a high amount of exercise was provided, suggesting that weight loss to low levels of fat mass may also mediate potentially adverse health effects. Overall, the findings of this thesis together with future studies may help to guide future exercise and dietary recommendations for normal-weight individuals (e.g., athletes, general population) wishing to aim for enhanced performance, aesthetic appearance, well-being, and longevity.

TIIVISTELMÄ

Nykypäivänä lukuisat ihmiset tavoittelevat parempaa ulkonäköä ja terveyttä noudattamalla tiukkoja ruokavalio- ja harjoitusohjelmia. Tiukoilla elämäntapoja muokkaavilla ruokavalio- ja harjoitusohjelmilla voi olla positiivisia, mutta myös negatiivisia vaikutuksia terveyteen. Systeemibiologian avulla kyetään objektiivisesti ja kattavasti arvioimaan muutoksia solutason metaboliassa, metaboliareiteissä ja niiden säätelyssä. Kehonkoostumuksen, liikuntaharjoittelun ja energiasaatavuuden muutoksien vaikutuksia systeemibiologiaan normaalipainoisten ihmisten osalta on tutkittu melko vähän tähän päivään mennessä.

Väitöskirja pyrkii täydentämään tietoa siitä, miten i) merkittävä painonpudotus ja painon uudelleenpalautuminen sekä ii) voimaharjoittelu ja siihen liittyvät kehonkoostumuksen muutokset vaikuttavat systeemibiologiaan entuudestaan terveillä normaalipainoisilla yksilöillä. Muutoksia systeemibiologiassa tutkittiin pitkittäisissä tutkimusasetelmissa tarkastelemalla muutoksia seerumin metabolomiikassa, plasman lipidiaineenvaihduntaa säätelevissä entsyymeissä ja proteiineissa, valkosolujen transkriptomiikassa, verisolujen määrissä, sytokiiniprofiilissa ja immunoglobuliini G:n glykosylaatiossa.

Naispuolisissa Fitness-kilpailijoissa (n=42) merkittävä kokonais- ja viskeeralirasvamassan väheneminen 20 viikkoa kestäneen intensiivisen harjoittelun ja energiavajeen seurauksena oli yhteydessä positiivisiin muutoksiin sydän- ja verisuonitautien riskitekijöissä, kuten seerumin triglyseridipitoisuuksiin, HDL-profiiliin ja tulehdustekijöihin. Jo ennen painonpudotusjaksoa kyseiset sydän ja verisuonitautien riskitekijät olivat suotuisammalla tasolla Fitness-urheilijoissa verrattuna iän ja painoindeksin suhteen kaltaistettuihin FINRISK-replikaatioaineiston naisyksilöihin (n=58). Painonpudotuksen havaittiin olevan myös yhteydessä vastaaviin positiivisiin muutoksiin sydän- ja verisuonitautien riskitekijöissä 7 vuoden seurannan aikana FINRISK-replikaatioaineistossa

(n=58). Fitness-urheilijoilla painonpudotusjakson aikaisen alhaisen energiasaatavuuden ja runsaan harjoittelun havaittiin lisäksi olevan yhteydessä immuunijärjestelmän ja sen säätelytoiminnan muutoksiin. Merkitsevät muutokset immuunijärjestelmässä sekä säätelevissä tekijöissä viittasivat muutoksiin erityisesti hematopoieesissa, valkosolujen erilaistumisessa sekä vasta-aine- ja sytokiinisignaloinnissa. Intensiivisellä painonpudotusjaksolla ei havaittu kuitenkaan pitkäaikaisia vaikutuksia sydän- ja verisuonisairauksien riskitekijöihin tai immuunijärjestelmään toimintaa, sillä valtaosa havaituista muutoksista palautui ennalleen painon ja rasvamassan palautuessa lähtölukemiin 20 viikon kuluessa.

Kuudentoista viikon voimaharjoitteluinterventio terveillä normaalipainoisilla nuorilla miehillä (n=59) oli yhteydessä merkitsevään lihasmassan lisääntymiseen ja rasvamassan vähentymiseen. Voimaharjoittelu yhdessä kehonkoostumusmuutosten kanssa oli yhteydessä myös suotuisiin muutoksiin seerumin metabolomissa, kuten IDL- ja LDL-lipoproteiinialaluokkien sekä apolipoproteiini B:n pitoisuuksien laskuun. Tutkimuksen perusteella yksilöt, joilla on heikoin lähtötilanne kehonkoostumuksen ja biomarkkeriprofiilin osalta näyttäisivät hyötyvän eniten säännöllisestä voimaharjoittelusta.

Väitöskirjan tulokset osoittavat, että suotuisat muutokset kehonkoostumuksessa jopa entuudestaan normaalipainoisilla yksilöillä harjoittelun ja/tai energiavajeen seurauksena ovat yhteydessä suotuisampaan sydän- ja verisuonitautien riskitekijäprofiiliin. Lisäksi, väitöskirja antaa uutta tietoa systeemibiologisista tekijöistä ja mekanismeista, jotka mahdollisesti välittävät immunosuppressiota ja immuunijärjestelmän säätelyn muutoksia pitkittyneen energiavajeen ja runsaan harjoittelun aikana. Väitöskirjan löydökset yhdessä uusien tutkimustulosten kanssa voivat auttaa tulevaisuudessa kehittämään ravinto- ja liikuntasuosituksia entuudestaan normaalipainoisille yksilöille, jotka tavoittelevat entistä esteettisempää ulkonäköä, parempaa terveyttä, suorituskykyä ja/tai pitkäikäisyyttä.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on **The Female Physique Athlete Study** and **The Male Resistance Training Study** from which the following publications were generated:

The Female Physique Athlete Study (Paper I and II)

I **Sarin HV**, Lee JH, Jauhiainen M, Joensuu A, Borodulin K, Männistö S, Jin Z, Terwilliger JD, Isola V, Ahtiainen JP, Häkkinen K, Kristiansson K*, Hulmi JJ*, Perola M*. Substantial fat mass loss reduces low-grade inflammation and induces positive alteration in cardiometabolic factors in normal-weight individuals. Scientific Reports **9,** 3450 (2019).

II **Sarin HV**, Gudelj I, Honkanen J, Ihalainen J, Vuorela A, Lee JH, Jin Z, Terwilliger JD, Isola V, Ahtiainen JP, Häkkinen K, Jurić J, Lauc G, Kristiansson K*, Hulmi JJ*, Perola M*. Molecular pathways underlying altered immunity in response to prolonged intensive physical training, low energy availability and substantial loss of fat mass. Front. Immunol. **10,** 907 (2019).

The Male Resistance Training Study (Paper III)

III **Sarin HV***, Ahtiainen JP*, Hulmi JJ, Ihalainen J, Walker S, Küüsmaa-Schildt M, Perola M, Peltonen H. Resistance training induces anti-atherogenic effects of metabolomic pathways associated with cardiovascular disease. Med Sci Sports Exerc. **51,** 1866–1875 (2019).

*Equal contribution.

CONTENTS

ABBREVIATIONS

1RM	one repetition maximum
APC	antigen presenting cell
BCR	B cell receptor
BMI	body mass index
BSA	body surface area
CLP	common lymphoid progenitor
CV	coefficient of variation
CVD	cardiovascular disease
DEG	differentially expressed gene
DILGOM	Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome
DEXA	dual-energy X-ray absorptiometry
FCRL	Fc receptor-like protein
FcεRI	high-affinity IgE receptor
FDR	false discovery rate
FFA	free fatty acid
FFQ	food frequency questionnaire
GC	germinal centre
GEE	generalized estimation equations
GlcNAc	N-acetylglucosamine
HILIC-SPE	hydrophilic interaction liquid chromatography solid-phase extraction
HSC	haematopoietic stem cell
IPAQ	international physical activity questionnaire
METh/wk	metabolic equivalent hour per week
MHC	major histocompatibility complex

MinDC	minimum detectable concentration
M0	macrophage progenitor
M1	classical macrophage
M2	wound-healing/regulatory macrophage
NK cell	natural killer cell
NMR	nuclear magnetic resonance
RCT	reverse cholesterol transport
ROS	reactive oxygen species
RT	resistance training
SD	standard deviation
SDS	sodium dodecyl sulphate
TG	triglyceride
T_{FH}	T follicular helper cell
T_H	T helper cell
THL	Finnish Institute for Health and Welfare
T2D	type 2 diabetes
Treg	regulatory T cell
TRL	triglyceride-rich lipoprotein
UPLC	ultra-performance liquid chromatography
WBC	white blood cell

The names of genes, metabolites, and cytokines are not included in this list of abbreviations.

1 INTRODUCTION

In the 21st century, humankind faces a new challenge with an environment promoting a sedentary lifestyle [1,2]. Reduced physical activity together with an abundance of available food are thought to be major causes for the growing incidence of non-communicable metabolic diseases that have superceded infectious diseases as the principal cause of death globally [3–5].

Lifestyle changes (e.g., weight loss, increased physical activity, healthier diet) have been shown to effectively rehabilitate the sedentary-lifestyle–related disrupted metabolic homeostasis and to improve future prognosis of morbidity [6]. However, it has not been studied in detail whether these beneficial effects of weight loss and increased physical activity can be generalized to the physiology of normal-weight individuals wishing to further promote well-being and longevity or whether adverse health effects can arise from engaging in intense exercise regimens and pursuing even lower levels of fat mass. Potentially, a significant public health issue as even higher number of individuals among normal-weight general population engage in vigorous exercise regimens, follow strict dietary plans, and try to rapidly lose weight in attempt to meet the general appearance standards set by modern society [7,8]. More studies are warranted on the effects of strict lifestyle regimens on the physiology of normal-weight individuals to discover whether these regimens have the potential to induce additional health benefits. More information is also needed to determine if these regimens have any adverse health effects [8] and to determine associated underlying molecular mechanisms often providing novel insight for general human (patho)physiology as well.

Further understanding of relationships between body composition, lifestyle factors (e.g., physical activity, nutrition), and associated molecular mechanisms can guide future lifestyle recommendations for normal-weight individuals on the general population and individual level. For example, despite the popularity of resistance training (RT) as a training method, little

evidence exists on how long-term RT modulates cardiovascular risk factors and participates in the prevention of chronic diseases in young and normal-weight individuals. This is a relevant topic, as a growing number of studies have highlighted the RT as an effective countermeasure to the development of age-related chronic diseases [9–13]. Increasing knowledge on these topics with the systems biology approach can help to identify new prognostic markers and to develop efficient preventive measures to be used in modern personalized medicine when combatting lifestyle-induced diseases.

Ongoing "omics era" with scientific advancements in the field of high-throughput screening methods have accelerated understanding of the complex systems biology underlying physiological changes associated with lifestyle changes [14,15]. With high-throughput screening methods used in this study, it is specifically focused on elucidating how adiposity compartments, muscular fitness, physical activity, and energy availability participate in the modulation of systems biological factors associated with cardiometabolic profile, immune-system function, and immune-system–mediated inflammation. These lifestyle factor associated pathways are investigated in longitudinal study settings in two Finnish study samples of healthy normal-weight individuals and in a subset of a body mass index (BMI)- and age-matched individuals from a Finnish population-based cohort [16–21].

2 REVIEW OF THE LITERATURE

2.1 Integrative systems biology of immune system and cardiometabolic signatures

Systems biology as an approach is able to elucidate complex molecular interactions and respective physiological entities by utilizing and integrating high-throughput screening methods [22]. By integrating various omics, this thesis focuses mainly on elucidating immune-system signatures, immune-system–mediated systemic inflammation, and lipid and lipoprotein metabolism-associated signatures.

2.1.1 Immune system

The immune system is a multifunctional ensemble consisting of adaptive and innate immune system responses, although characterised as separate entities, they work in unison to distinguish self from non-self and to defend individuals from a universe of pathogens, allergenic, and toxic substances [23,24]. Essentially, the innate and adaptive immune systems consist of physical barriers and synergistic interplay between immune-system cells and secreted signalling molecules (Figure 1). In response to foreign invaders and damaged tissue, the immune system is also programmed with the ability to initiate inflammation to restore homeostasis.

2.1.1.1 Haematopoiesis and proliferation of immune-system cells

All adaptive and innate immune-system cells originate from haematopoietic stem cells (HSCs) located in red bone marrow – mainly situated in the central skeleton (e.g., pelvis, sternum, cranium, ribs, vertebrae, and scapulae), whereas proliferation, differentiation, and activation of immune cells occurs in circulation and peripheral immune system organs (e.g., spleen, thymus, lymph nodes). In red bone marrow, HSC lineage fate and proliferation are tightly regulated by multiple signals from regulating transcription factors and signals from circulation mediated by cytokines,

chemokines, and hormones [25]. Choice of HSC lineage fate and main regulatory signals from transcriptional factors are illustrated in Figure 2.

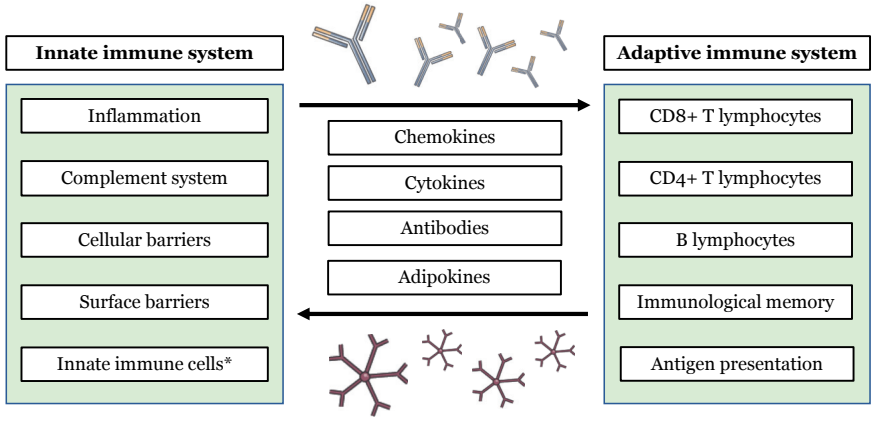


Figure 1. Overview of adaptive and innate immune system components. * Innate immune cells include mast cells, eosinophils, basophils, macrophages, natural killer (NK) cells, NK T cells, dendritic cells, neutrophils, and monocytes. An illustration website (https://smart.servier.com) was utilized in figure production.

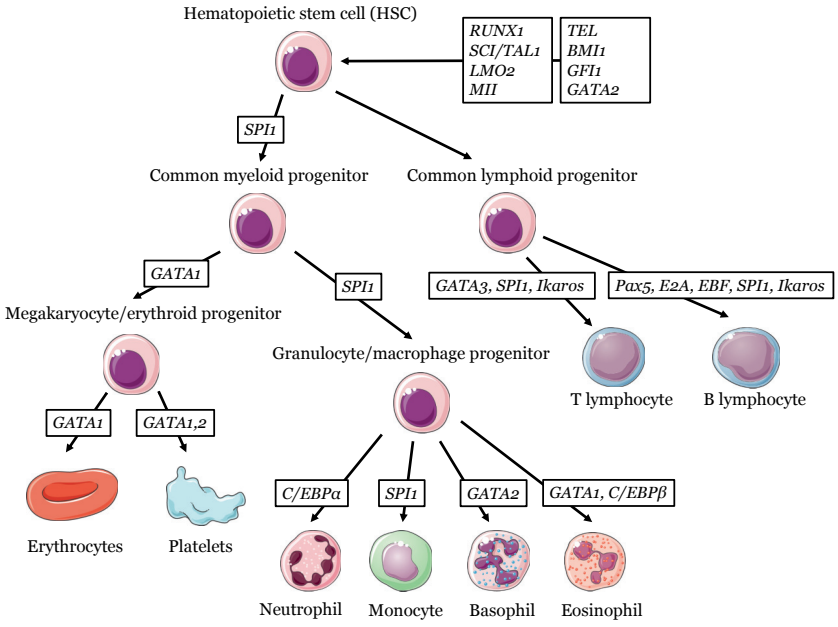


Figure 2. Illustration of HSC lineage fates and stepwise transcriptional regulation needed for HSC proliferation to distinct immune cell lines. HSC proliferation regulating gene names are depicted with *italics* within boxes. Figure is modified from [25,26]. An illustration website (https://smart.servier.com) was utilized in figure production.

2.1.1.2 Innate immune system

The innate immune system stems from an older evolutionary background compared to the adaptive immune system [24]. The innate immune system consists of physical barriers (e.g., skin, mucosa), the complement system, ability to initiate inflammation, and white blood cells (WBCs) [e.g., mast cells, macrophages, monocytes, neutrophils, dendritic cells, basophils, eosinophils, natural killer cells (NKs), and NK T cells] as demonstrated in Figure 1. For the purposes of this thesis, relevant literature on the innate immune system associated WBCs will be reviewed in detail.

Monocytes and macrophages

Monocytes play an important role in immune defence, inflammation, and homeostasis by sensing their local environment, clearing pathogens and dead cells, and initiating adaptive immunity [27]. Circulating monocytes can be divided into at least three subsets — classical, non-classical, and intermediate monocytes. Classical monocytes have been characterised by high antimicrobial capability by secreting high volumes of proinflammatory cytokines and reactive oxygen species (ROS). Intermediate monocytes cytokine profile resembles the classical monocyte, whereas non-classical monocytes secrete only small quantities of cytokines.

Monocyte activation leads to tissue infiltration and macrophage formation [27]. Depending on the cytokine microenvironment and associated immune cells, monocyte macrophage progenitors (M0) have the ability to differentiate into classical (M1), wound-healing (M2), or regulatory macrophages (M2) as shown in Figure 3. M1 macrophages are highly microbicidal, mediate CD4 T_H1 cell responses, and promote tumour suppression. On the other hand, M2 macrophages are considered immunosuppressive as they induce T_H2 responses, wound healing and tissue repair, and tumorigenesis.

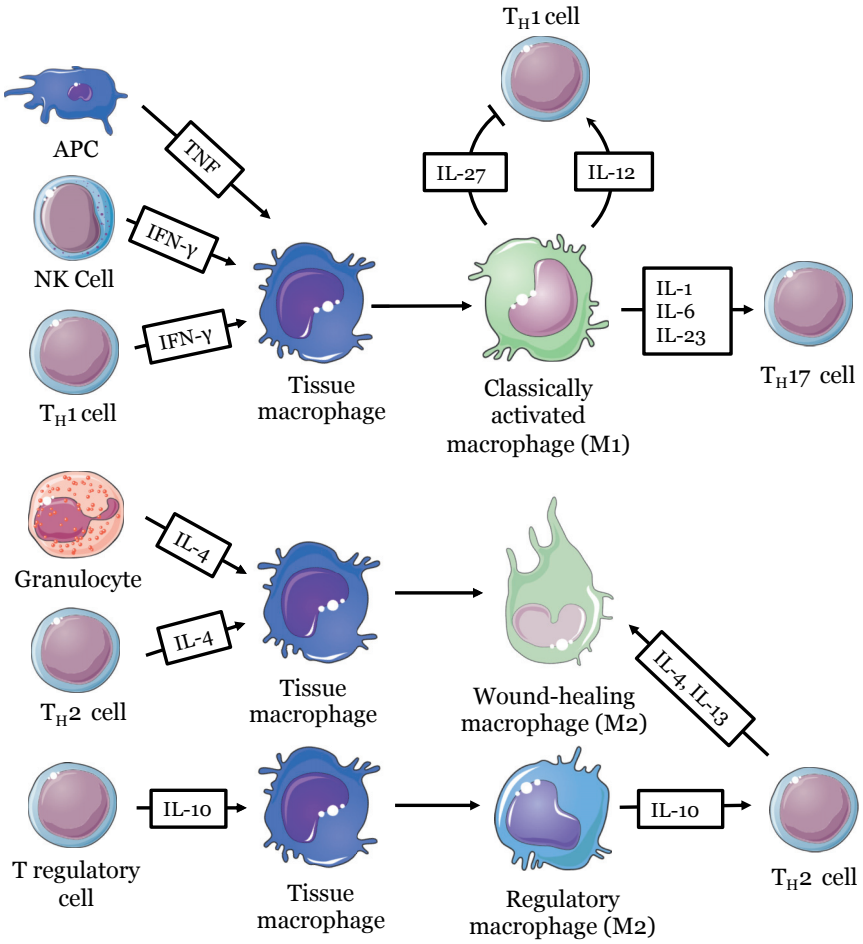


Figure 3. Macrophage proliferation and interactions with other immune cells. Arrows indicate positive feedback while bar-headed lines indicate negative feedback regulation. Figure is modified from [27]. APC = antigen presenting cell. NK cell = natural killer cell. T_H cell = T helper cell. TNF = Tumour necrosis factor. IFN = Interferon. IL = Interleukin. An illustration website (https://smart.servier.com) was utilized in figure production.

Granulocytes

Granulocytes, also known as polymorphonuclear leukocytes, are innate immune cells characterised by the presence of granules in their cytoplasm. Granulocyte subsets include neutrophils, eosinophils, basophils, and mast cells, and they arise from granulocyte/macrophage progenitors from red bone marrow as demonstrated in Figure 2.

Neutrophils are the most abundant granulocyte subpopulation in circulation [28]. They are professional phagocytes that target pathogens coated with antibodies and complement, as well as damaged cells or tissue. Neutrophils influence not only the multiple aspects of the inflammatory and immune responses, but also tumour development, antiviral defence, haematopoiesis, angiogenesis, and fibrogenesis.

The major functions of eosinophils in the human immune system include combatting multicellular parasites and infections, regulation of other immune cell functions, and control mechanisms associated with allergy and asthma [29]. In addition, eosinophils function as an effector arm of T_H2 mediated immunity [30]. Similar to eosinophils, basophils participate in the formation of acute and chronic allergic responses as well as inflammatory reactions during immune responses [31]. Basophils express high-affinity immunoglobulin E (IgE) receptors, Fc epsilon receptor (FcεRI), on their cell surface that bind IgE, an immunoglobulin involved in primarily pathogen defence and allergic reactions. Subsequently, eosinophils are also effectors of allergic inflammation as mentioned earlier and partner with mast cells to respond to elevations in IgE.

Mast cells are only present in tissues where they mediate host defence against pathogens, allergic reactions, inflammation, and autoimmunity, wound healing, angiogenesis, and blood-brain barrier function [31]. Mast cell characteristics of appearance and functions are very similar to basophils with i) mast cells granules containing histamine/heparin and ii) IgE mediated humoral immunity through high-affinity receptors, FcεRIs.

2.1.1.3 Adaptive immune system

The adaptive immune system is composed of sophisticated defences with mainly highly specialized immune cells, lymphocytes, that are specific to particular pathogens and foreign invaders [32]. As depicted in Figures 1 and 2, lymphocytes can be divided into two broad classes based on the immune response type – B cells (antibody-mediated) and T cells (cell-mediated). Cell

line commitment, proliferation, and associated regulatory networks of these two lymphocyte cell lines are reviewed in detail in the following chapters.

Regulation of B cell line commitment and specification

As the sole source of immunoglobulins, B cells are an essential component of the adaptive immune system [33]. Early forms of B cells arise from common lymphoid progenitors (CLP) under the regulation of numerous transcriptional factors and surface proteins in the bone marrow as demonstrated in Figure 2. Lymphoid specification and commitment of CLPs is a gradual process that is characterised with the stage-like expression of specific receptors and markers located on the B cell surface (Figure 4) [33]. Subsequently, the expression of key transcription factors is also required as

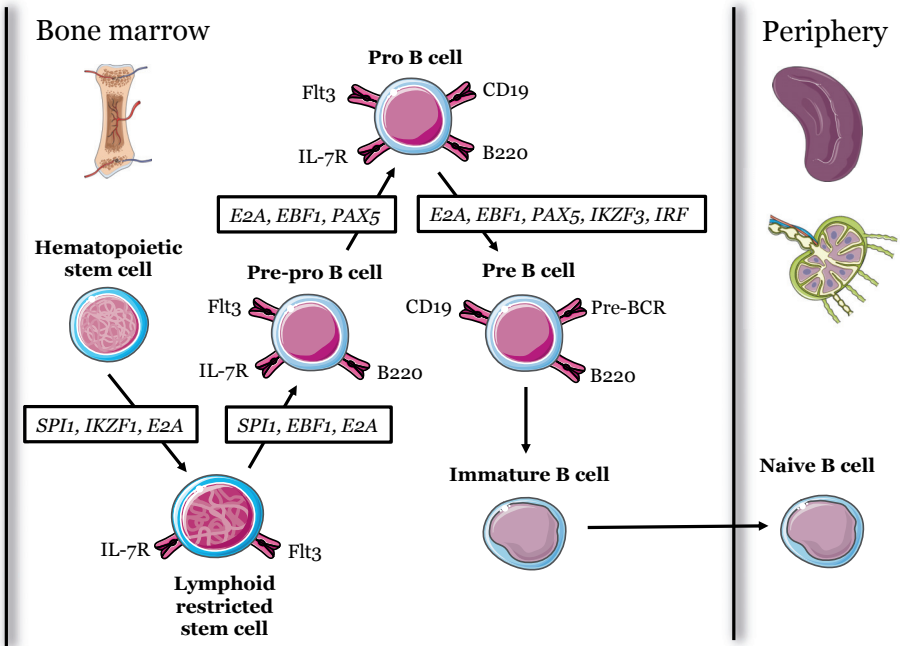


Figure 4. Overview of the B cell lineage commitment in bone marrow. Figure is modified from [33]. Regulating transcription factors and genes have been depicted with boxes, whereas cell surface receptors with receptor illustrations. Genes and transcription factors are designated with *italics* while proteins are not. An illustration-website (https://smart.servier.com) was utilized in figure production.

they work in unison to mediate activator/repressor signalling to the cell surface proteins in a coordinated action that is critically essential for the formation of functional B cells (Figure 4) [33].

Transcriptional regulation of B cell proliferation

B cells need to undergo further maturation in peripheral lymphoid tissues, where they attain distinct features needed for effective immune system function. There, B cells can differentiate into memory B cells or antibody secreting plasma cells that can occur through the germinal centre (GC) pathway (and by marginal zone) (Figure 5) [34]. Peripheral B cell maturation and proliferation is dependent on transcriptional regulation and signalling by B cell receptors (BCR), antigen presenting cells (APCs), and CD4+ T helper cells (T_H).

GCs are sites within peripheral lymphoid organs (e.g., lymph nodes or spleen) where B cells proliferate and differentiate through somatic hypermutation of genes and antibody class switching (Figure 5) [34]. Thus, GCs have a key role in the B cell humoral immune response by acting as central factories for the generation of affinity matured B cells specialized in producing improved antibodies that effectively recognize foreign pathogens and substances. Subsequently, the expression of key transcription factors together with follicular helper T (T_{FH}) cell specific selection is required [34]. Detailed characteristics of the B cell proliferation transcriptome and GC sites are reviewed in Figure 5.

Antibody mediated immunity

Antibody mediated immunity is controlled by plasma cells that have the ability to produce five different antibody categories IgG (75%), IgE (0.05%), IgA (15%), IgD (0.25%), and IgM (10%). IgG antibody is the most abundant antibody in the human circulation that is released by plasma cells. In general, antibodies are major regulators of humoral immunity where IgGs have several functions, including pathogen opsonization, classical pathway

of complement system activation, toxin neutralization, functions in antibody-dependent cell-mediated cytotoxicity, and functions in type II and III hypersensitivity reactions.

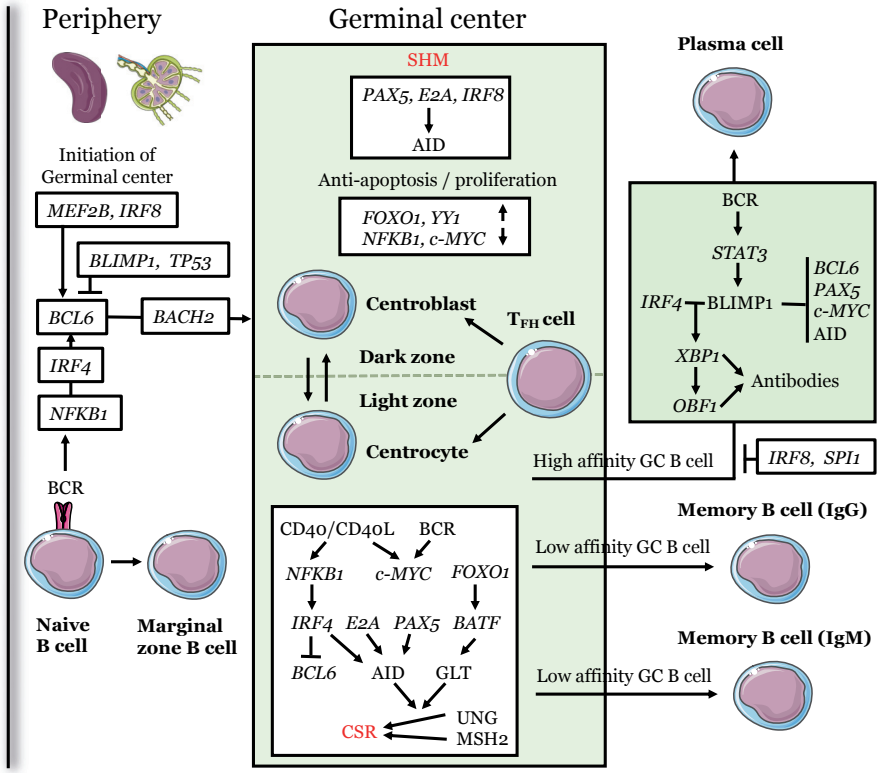


Figure 5. B cell proliferation and transcriptional regulation in the periphery to antibody secreting plasma cells and/or memory cells. Genes and transcription factors are designated with *italics* while proteins are not. Figure is modified from [34]. SHM = somatic hypermutation. CSR = class switch recombination. BCR = B cell receptor. Arrows = activation. Bar-headed lines = inhibition. An illustration website (https://smart.servier.com) was utilized in figure production.

Effective humoral response by IgG requires adequate IgG glycosylation and formation of the two existing binding sites. Differential IgG glycosylation of N-glycans has been shown to modulate complement activation thus modulating inflammation status and antibody affinity with Fc like receptors (FCRLs) [35,36]. Differential expression transcription factors

(e.g., *RUNX3*, *IKZF1*) and genes (e.g., *ST6GAL1*, *B4GALT1*, *MGAT3*, FUT8) regulating associated enzymatic processes are known to affect glycosylation significantly, thus influencing the affinity and binding capacity of IgGs [37]. Furthermore, IgG binding sites also have major effects on IgG binding affinity with antigens, as the antigen binding site is formed by the variable domain of one heavy chain, together with its associated light chain that are produced by respective genes.

T cell characteristics and line commitment

In a similar manner as B cells, after leaving the bone marrow, mobilized immature T cells are transported to peripheral lymphoid tissues, primarily thymus, for further proliferation (Figure 6). Peripheral T cell proliferation produces T cell subclasses with different cell-surface glycoprotein, namely CD4+ and CD8+ [38]. Furthermore, CD4+ cells have the ability to differentiate into subsequent effector subgroups, namely regulatory T cells (Tregs) or number of CD4+ T_H subsets – T_H1, T_H2, T_H17 and T_{FH}, whereas CD8+ cells differentiate primarily to CD8+ cytotoxic cells [39,40]. T cells play a critical role in cell-mediated immunity and are characterised by cell-surface T cell receptors.

Transcriptional regulation of T cell proliferation

Of the several transcription factors reported as of importance for early T cell development — the Notch signalling pathway is one of the most essential (Figure 6) [38]. Lineage fate choice between CD4+ T_H cell and CD8+ cytotoxic T cells is under tight regulation by numerous transcription factors and APCs. T cell subsets are antigen specific as CD4+ are restricted to major histocompatibility complex (MHC) I, whereas CD8+ development is induced by MHC II conjugation.

The cell-mediated immune response modulation is tailored by the differentiation of CD4+ T_H cells into aforementioned distinct effector cell groups, promoted by antigen presentation, the balance of cytokine profile,

and subsequent stimulation (Figure 7) [38]. However, generic categorization of CD4+ effector cell groups has been proven to be an oversimplification of CD4+ cell proliferation and function as the reversible plasticity of CD4+ subsets enables a great deal of differentiation variation depending on the microenvironment (Figure 7) [40].

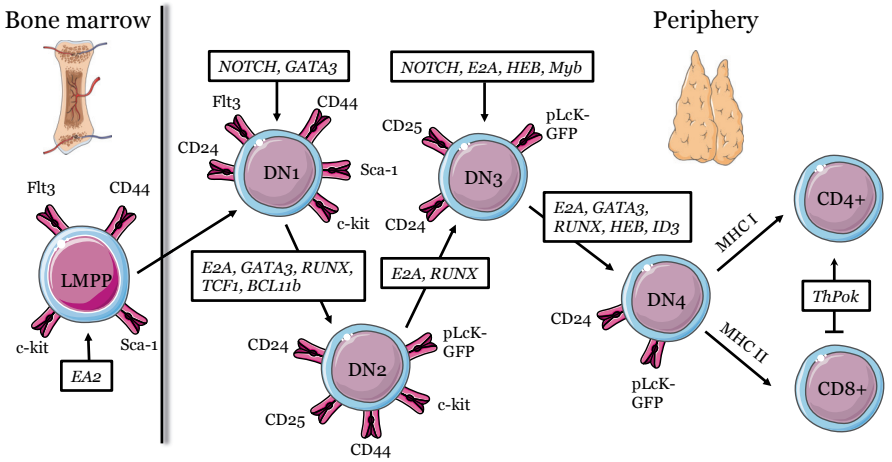


Figure 6. Illustration of key transcription factors participating in the regulation of T cell proliferation. Modified from [38]. Transcription factors are presented in boxes, proliferation stage inside illustrated thymocytes, and cell surface receptors with receptor illustrations. LMPP = lymphoid-primed multipotent progenitor. DN = double-negative. MHC = major histocompatibility complex. An illustration website (https://smart.servier.com) was utilized in figure production.

2.1.2 Inflammation and Immune system

Inflammation and acute phase response are essential complex biological processes that protect an organism against pathogens and tissue damage by inducing a transient coordinated accumulation of blood cells, cytokines, and chemokines. The inflammation response focuses in restoring host homeostasis by repairing damaged tissue and removing foreign invaders [41]. The immune system plays a key role as an inflammation instigator by introduction and activation of leukocytes together with vascular vasodilation. Activation of leukocytes promotes the secretion of various inflammation mediators, such as cytokines, chemokines, and proteolytic

cascade products. These inflammatory mediators attract other immune systems cells, including lymphocytes, monocytes, and neutrophils, that participate and promote the clearance of inductor antigens [41].

A transient acute inflammatory response is considered beneficial for the host, whereas a prolonged low-grade inflammation is detrimental for physiological homeostasis and health. Low-grade inflammation is characterised by increased levels of circulating proinflammatory markers (e.g., C-reactive protein (CRP), TNF-α, IL-6, α_1-acid glycoprotein, IP-10, leptin), chronic elevation in leukocytes, and tissue malfunction. Chronic low-grade inflammation is associated with increasing age, sedentary lifestyle, smoking, obesity, and subsequently with chronic non-communicable diseases including type 2 diabetes (T2D) and cardiovascular disease (CVD) [42–45].

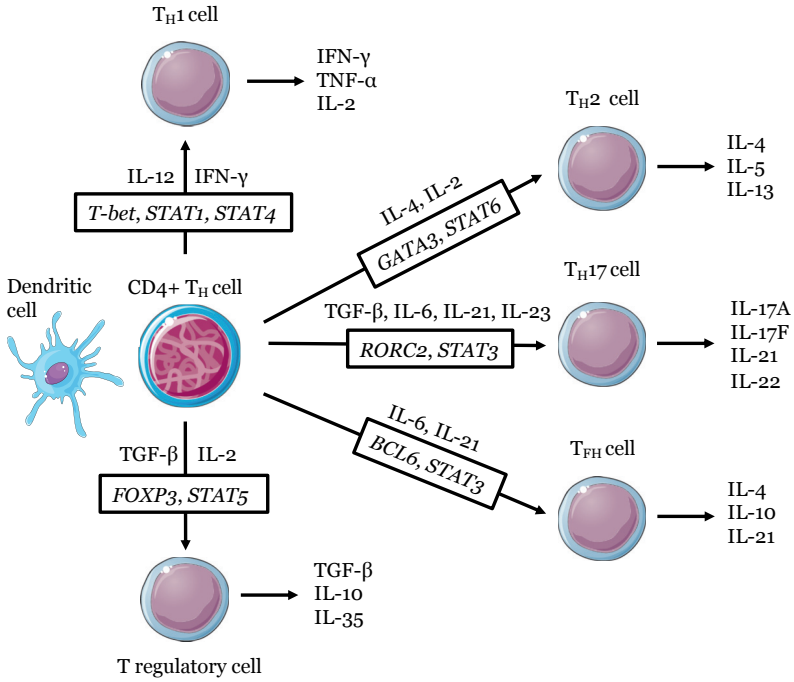


Figure 7. Transcriptional and cytokine regulation of CD4+ T_H cell proliferation to different effector groups. Modified from [39,40]. Transcription factors are depicted within boxes, while contributing cytokines are not. IL = interleukin. TNF = tumour necrosis factor. IFN = interferon. TGF = tumour growth factor. T_H cell = T helper cell. An illustration website (https://smart.servier.com) was utilized in figure production.

2.1.3 Lipid and lipoprotein metabolism

Lipid metabolism is a complex physiological entity where tightly regulated homeostasis between intestinal absorption, enterohepatic circulation, peripheral mobilization, and oxidation is essential for cardiovascular health. Disturbances in lipid and lipoprotein metabolism resulting in dyslipidemia are major risk factors for CVD [46].

Peripheral enzyme activity is essential for lipid transport between lipoproteins [e.g., high-density lipoprotein (HDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), very-low-density lipoprotein (VLDL)] and lipoprotein lipid distribution. Major key regulating enzymes modulating lipoprotein distribution and content are lecithin–cholesterol acyltransferase (LCAT), phospholipid transfer protein (PLTP), paraoxonase-1 (PON1), cholesteryl ester transfer protein (CETP), and angiopoietin-like proteins 3,4, and 8 (ANGPTL) [47–52]. Composition of different lipoproteins varies regarding protein, cholesterol, cholesterol ester, phospholipid, and triglyceride (TG) content. The lipoprotein with the highest density, HDL, possesses the most abundant amount of proteins and phospholipids together with the lowest concentration of TGs. Conversely, lower density lipoproteins, LDL, IDL, and VDL, possess more TGs and cholesterol together with less protein and phospholipid content. A schematic overview of peripheral lipoprotein metabolism is depicted in Figure 8.

Reverse cholesterol transport (RCT)

The reverse cholesterol efflux pathway mediates cholesterol transport from the periphery to the liver and subsequently to bile that is excreted into the intestinal lumen and stool. Reverse cholesterol efflux is initiated by nascent discoidal HDL that is formed by liver produced apolipoprotein (apo) A1 together with serum phospholipids (Figure 8) [53]. These particles interact with the peripheral cholesterol receptor ABCA1 through which cholesterol is secreted from peripheral cells (e.g., macrophages, fibroblasts) to produce nascent preβ HDL particles. ABCA1-mediated RCT pathway may in turn

promote cholesterol efflux via receptors ABCG1 and SR-BI and other related pathways [54].

LCAT is an enzyme bound to mainly HDLs where it transforms free cholesterol into cholesteryl ester, which is then sequestered into the core of a lipoprotein particle, thus eventually making the new synthesised HDL spherical and larger (Figure 8) [47]. LCAT is an important facilitator of RCT efflux pathway by modulating HDL content and size. Removal of free cholesterol from the surface of HDL enables the reaction to become unidirectional and facilitates additional cholesterol binding, thus promoting cholesterol removal from peripheral cells.

CETP may further enhance this process of cholesterol binding to HDLs by transferring cholesteryl esters formed by LCAT from HDL onto apoB100-containing lipoproteins in exchange for TGs, thus making HDLs even more susceptible to cholesterol binding (Figure 8) [55]. Subsequently, apoB100-containing lipoproteins transport these cholesteryl esters to the liver via low-density-lipoprotein receptor (LDL-R) to be excreted.

Moreover, PLTP also works in unison with LCAT to produce larger HDL particles by transferring phospholipids from TG-rich lipoproteins (TRLs) to HDLs and by fusing smaller HDL_3 together to produce HDL_2 [48]. End products, large HDL_2 particles, possess apoA1 that is essential for HDL uptake by SR-B1 receptors to the liver, where cholesterol from HDL can either be secreted via the bile to the intestine or be used to synthesise steroid hormones.

Lastly, HDLs also possess PON1 on their surface, which protects against macrophage-mediated LDL oxidation, and increases HDL binding to macrophages which, in turn, stimulate HDL's ability to promote cholesterol efflux (Figure 8) [56].

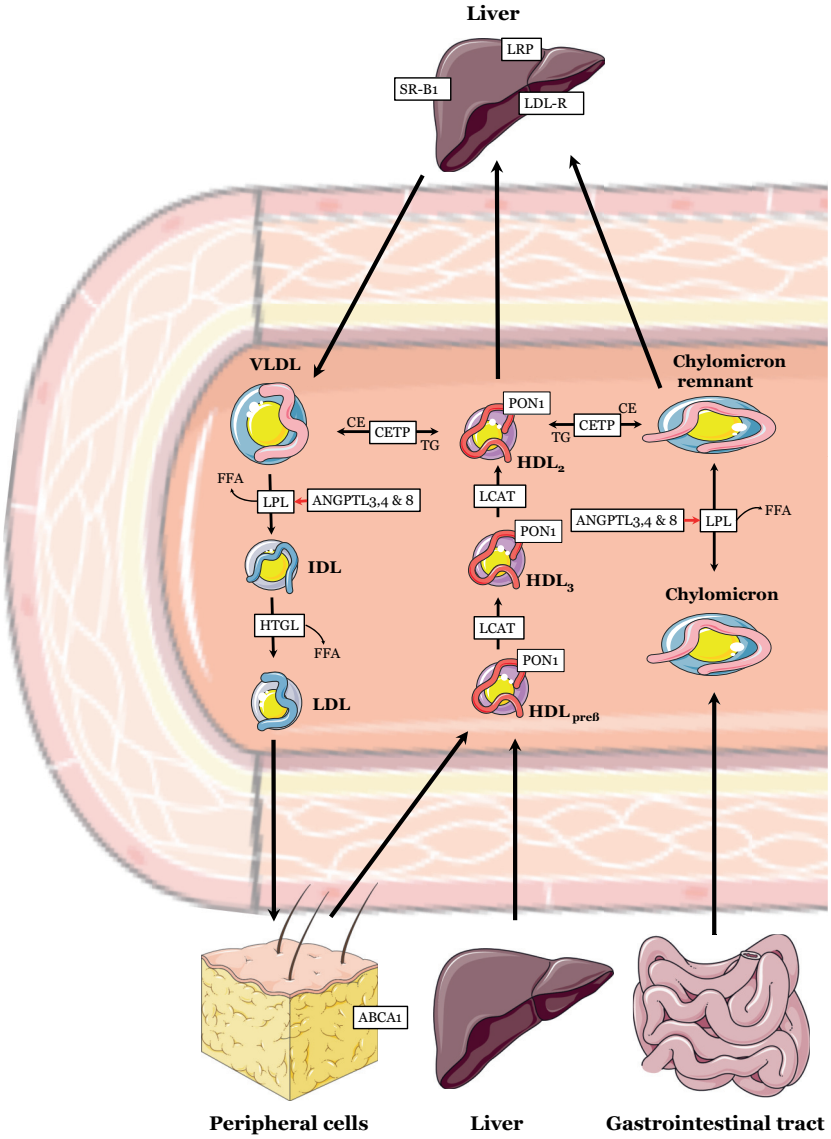


Figure 8. Overview of lipoprotein metabolism between peripheral tissues, gastrointestinal tract, liver, and circulation. TG = triglyceride. CE = cholesterol ester. FFA = free fatty acid. HTGL = hepatic triglyceride lipase. LCAT = lecithin–cholesterol acyltransferase. PLTP = phospholipid transfer protein. PON1 = paraoxonase-1. CETP = cholesteryl ester transfer protein. ANGPTL = angiopoietin-like protein. LRP = low-density lipoprotein receptor-related protein. SR-B1 = Scavenger receptor class B type 1. LDL-R = low-density lipoprotein receptor. Red arrows indicate regulatory effect of ANGPTLs towards lipoprotein lipase (LPL) activity. Receptors and enzymes/proteins that participate in the regulation of lipoprotein metabolism are indicated within boxes. An illustration website (https://smart.servier.com) was utilized in figure production.

Exogenous cholesterol pathway

The exogenous cholesterol pathway is responsible for producing TRLs from liver to be transported to the periphery. First, VLDLs consisting mainly of TGs are secreted from the liver to the circulation [57]. There, in a stepwise manner, lipoprotein lipase (LPL) mobilizes TGs from the surface of VLDL as free fatty acids (FFAs) that can be utilized as an energy source in peripheral tissues, such as muscle tissue or stored into adipose tissue (Figure 8). Thus, active LPL function degrades VLDL TG content and reduces the TG/protein ratio, therefore resulting first in the formation of IDLs and then LDLs (Figure 8). Subsequently, hepatic triglyceride lipase also participates in the process of transforming IDL to LDL by dislodging FFAs. Characterised LPL function is of importance for effective TG clearance from circulation and preventing the formation of hypertriglyceridemia. More recently characterised ANGPTL-3, -4, and -8, inhibitors of LPL activity, have the ability to enhance or suppress TG clearance (Figure 8) [57]. Mature LDL particles have two possible fates as they can be taken up by LDL-receptor (LDL-R) in the liver for secretion or steroid formation, whereas in the periphery LDL is transported to cells by receptor-mediated endocytosis.

Endogenous cholesterol pathway

The key role of the endogenous cholesterol pathway is to transport TG-rich chylomicrons from intestinal enterocytes to the circulation and to the liver [57]. Enterocyte-derived nascent chylomicrons consist mainly of TGs, together with cholesterol, and apoA1 and apoB48. Maturation from nascent chylomicrons to chylomicrons quickly cleaves the apoA1 that is transferred to HDL particles. In exchange, chylomicrons receive apoE. After apolipoprotein exchange, in a similar manner as with VLDL, LPL participates in mobilization of chylomicron TGs to FFAs and subsequently promotes the formation of chylomicron remnants with more diminished TG content. These chylomicron remnants can be taken up by LDL-receptors

(LDL-R, LPR) in the liver. Furthermore, CETP can enhance TG depletion of chylomicron remnants by substituting TGs with cholesterol esters derived from HDL_2, thus demonstrating the existing link between the RCT pathway and endogenous cholesterol pathway (Figure 8) [57].

Free fatty acid level regulation and metabolism

Fatty acid metabolism consists of both catabolic processes that generate ATP, and anabolic processes that create biologically important signalling molecules (e.g., diacylglycerols, ceramides, eicosanoids) [58]. These actions, together with dietary intake of fatty acids, contribute to circulating levels and distribution of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in the circulation.

During fasting, FFAs are released from adipose tissue, chylomicrons, and lipoproteins by LPL to the circulation, where they bind to carrier molecules such as albumin. This catabolic process is initiated by augmented levels of hormones, epinephrine and glucagon, and reduced levels of insulin. FFAs attached to albumin are mobilized into human circulation from which fatty acid transport proteins (FATPs, CD36) facilitate the uptake of fatty acids into peripheral cells [57]. After transportation into cells, fatty acids can be shuttled further into mitochondrial matrices via a carnitine shuttle pathway and used as an energy source in a stepwise process called beta-oxidation spiral. To some extent, FFAs can also be transported to peroxisomes for oxidation [59].

After consuming a meal, fatty acid synthesis is promoted by opposite shifts in the levels of aforementioned hormones that also regulate beta-oxidation [60]. Fatty acid synthesis occurs via the six recurring reactions, until the production of 16-carbon palmitic acid, that can further be elongated and/or desaturated by catalysing enzymes. High levels of palmitoyl-CoA, the final product of SFA synthesis, prevents the build-up of fatty acids in cells, whereas citrate from the Krebs cycle acts to activate fatty acid synthesis [61]. Subsequently, palmitoyl-CoA also has the ability to inhibit fatty

acids from associating with carnitine by regulating the enzyme carnitine acyltransferase, thereby preventing them from entering the mitochondria and beta-oxidation spiral.

Lastly, as the human body has a limited ability to produce unsaturated longer-chain ω-3 fatty acids — eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) — they need to be mostly obtained from the diet [62].

2.1.4 Amino acid metabolism

All of the 20 amino acids necessary for human health can be categorized primarily as non-essential, conditionally essential, and essential amino acids (Figure 9). While the non-essential amino acids can be synthesised using already existing intermediates, the essential amino acids need to be ingested in the diet. Quantity and distribution of amino acids in circulation is effectively a reflection of dietary intake, protein turnover (e.g., breakdown, synthesis, secretion), and oxidation for energy [63]. Dynamic changes in the amino acid pool occur by catabolic and biosynthetic pathways, as illustrated in Figure 9.

Dietary protein and influx of absorbed amino acids has the greatest impact on the circulating levels of amino acids, while body protein degradation, and amino acid synthesis influence is limited. Protein rich diet, and excess supply of amino acids in relation to the body's needs for protein synthesis, results in catabolism of the excess proteins. Following protein catabolism, degradation of amino acids can facilitate the production of intermediates used as precursors for energy production or other non-protein nitrogen compounds such as hormones, neurotransmitters, and creatinine [63].

Based on the type of intermediates produced during their breakdown, amino acids can be classified as being glucogenic and/or ketogenic amino acids [63]. The breakdown of glucogenic amino acids produces either pyruvate or other Krebs Cycle intermediates, while the breakdown of ketogenic amino acids produces acetyl-CoA. These

intermediates can be either oxidized in the Krebs cycle or used as precursor in the biosynthesis pathways producing glucose and FFAs. Overall, depending on the metabolic demands, amino acids can be considered as "biochemical multipotent molecules" able to be converted into energy, carbohydrates, lipids, or biochemical intermediates [64]. Lastly, it should be noted that these mechanisms of amino acid metabolism are tightly regulated, thus ultimately resulting in remarkably constant amino acid levels [65].

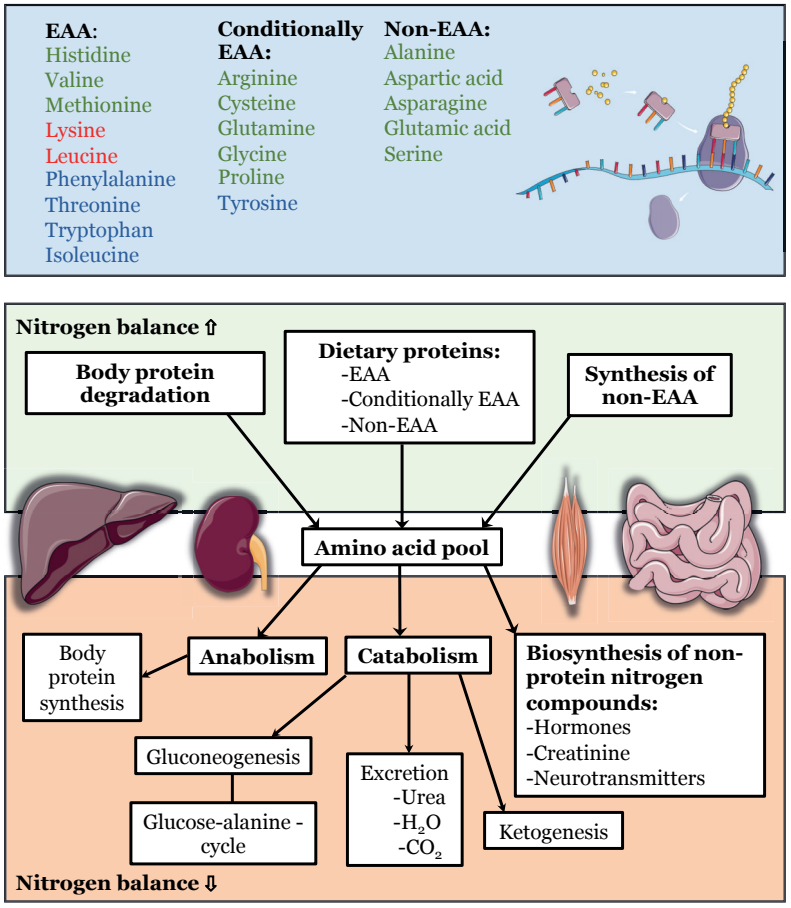


Figure 9. Amino acid metabolism. EAA = essential amino acids. Non-EAA = non-essential amino acids. Amino acid colouring in the upper panel indicates: green = glucogenic amino acids, red = ketogenic amino acids, and blue = keto/glucogenic amino acids. An illustration website (https://smart.servier.com) was utilized in figure production.

The whole body protein pool, as well as that of individual tissues, is determined and regulated by interactions among various factors including hormonal, nutritional, neural, inflammatory, and other influences [64]. Essentially, a key regulator of the homeostatic condition of amino acid biosynthesis and degradation is the mammalian target of rapamycin complex 1 (mTORC1) [65]. Under dietary deficit (i.e., amino acid depletion), mTORC1 inactivation increases the breakdown of cellular proteins through autophagy and reduced protein biosynthesis [65]. In contrast, food intake briefly increases plasma amino acid levels, which stimulates mTOR-dependent protein synthesis. Specifically, of all amino acids, leucine is considered as the primary nutritional regulator of muscle protein anabolism due to its ability to trigger the mTOR pathway [66].

2.2 Body composition, exercise training, and energy availability as determinants of immune system and cardiometabolic signatures

The roles of body composition, exercise training, and energy availability as determinants of metabolic health and future risk of non-communicable disease have been well established in the past in the overweight population [6,67–69]. However, it is still unknown whether the systems biology of normal-weight healthy individuals responds to these modalities in a similar manner, thus further promoting well-being and health. It has been suggested that these phenotype characteristics and lifestyle factors follow a U-shaped trend in mediating health effects where extremely low levels of fat mass, high levels of exercise training, and energy deprivation may lead to adverse health outcomes [70–72]. Next, key modalities of this thesis (i.e., physique competing, RT) is briefly discussed before reviewing the current state of knowledge on how different body composition compartments, exercise bouts and training, and energy availability are known to alter systems biological factors and respective physiological entities investigated in this thesis.

Fitness/physique competing

Fitness sports (i.e., physique sports), which are judged on muscularity, leanness, and aesthetic appearance, require intense weight reduction by combining high volumes of aerobic exercise training and RT with low energy intake preceding competitions [73]. Low energy availability is in general achieved by increasing the amount of aerobic exercise training and through energy deficit from diet while frequency and intensity of RT is kept at a relatively constant level to minimize the amount of muscle mass lost. Thus, athletes participating voluntarily in such activities are ideal study participants for assessing the physiological changes associated with weight loss in a population of very active and healthy normal-weight individuals. It has been speculated that physique competing associated severe loss to low levels of fat mass might predispose to adverse modulation of physiology, and health outcomes such as thyroid dysfunction, menstrual irregularities, and loss of bone mass [73,74]. However, to this date, the evidence is scarce on the acute and long-term health outcomes of physique competing associated substantial weight loss.

The vigorous 2-5-month progressive competition weight loss routine is usually followed by a voluntary weight-regain period, during which exercise training and energy intake levels are reverted back to normal levels [73]. This subsequent weight regain has been considered mandatory and beneficial to restore potentially disrupted metabolic homeostasis caused by prolonged low energy availability, intense exercise training, and extremely low levels of fat mass. During periods of off-season that i) follow after the weight-regain period from a competition, or ii) precede the first competition, physique athletes maintain healthy dietary routines with moderate energy surplus and RT dominant exercise routines while aiming to gain lean mass and refine their muscular symmetry. Although, obesity and weight gain in general have been considered detrimental for overall health, it is yet to be determined whether weight (re)gain from low levels of

fat mass and weight cycling within a normal-weight range affects human physiology and health in a similar manner [75].

Resistance training

Overall, RT is characterised as any exercise that causes the muscles to contract against an external force with the expectation of increases in muscle strength, muscle hypertrophy, and/or endurance abilities [76,77]. Subsequently, it has been shown that engaging in RT induces neurological and morphological changes contributing to increased voluntary muscle activation and fibre size that result in the development of muscle hypertrophy and strength. The physiological demands and outcomes of RT depend on the training variables, including mode (eccentric and/or concentric), volume, load, rest periods, and intensity of RT [76].

RT has been shown to promote muscle hypertrophy, muscle strength, functionality in daily life, and overall health [9]. Benefits of RT have been proven, especially in the elderly population, where RT delineates muscle mass loss, sustains functionality in everyday life, increases quality of life, and improves cognitive function [10–12]. However, the effects of regular RT on the overall health of healthy normal-weight young adult population have not been investigated in detail. In the adult population of modern society, RT and associated modalities, such as physique competing and CrossFit, have gained popularity as tools to pursue muscularity, leanness, and aesthetic appearance. Although, RT has been associated with a beneficial modulation of body composition through increased muscular hypertrophy — RT has not been convincingly associated with clinically significant weight loss in the past [78]. This is despite the fact that RT-induced muscular hypertrophy and increased lean mass results in the slight elevation of resting metabolic rate, thus potentially promoting body weight regulation and prevention of fat mass (re)gain.

2.2.1 Immune system signatures

Haematopoietic regulation and blood cell distribution

Haematopoiesis and the regulation of HSC pool proliferation in bone marrow is affected by lifestyle factors and physiological characteristics, including adiposity, exercise training, energy availability, and aging together with hormonal balance. Homeostasis of haematopoietic regulation is essential as dysregulated haematopoiesis can lead to adverse alteration in circulating blood cells (e.g., anaemia, leukocytosis, leukopenia) and associated immune system function and performance.

Increased adiposity and obesity [body mass index (BMI) > 30] are known to cause a marked increase in the size and number of adipocytes encroaching into the bone marrow space, thus leading to disruption of the haematopoietic environment and HSC interactions [79]. A similar increase in bone marrow adiposity can be also caused by other metabolic disorders - conversely associated with low energy availability such as anorexia nervosa, osteoporosis, and aging [80,81]. The aforementioned metabolic disturbances promote myeloid-skewed proliferation of HSC niches and predispose to systemic inflammation by inducing leukocytosis, namely neutrophilia [80].

Potential disruption of HSC niche homeostasis in obesity is also associated with higher levels of circulating platelets, that can together with leukocytosis contribute to the pathogenesis of CVD through inflammatory activation and aggregation processes [82]. Interestingly, acute strenuous exercise also has the potential to increase platelet count and enhance platelet aggregability, whereas continuous exercise training has been shown to reduce platelet adhesiveness and aggregability [83,84]. In the past, energy deficit observed in both intentional weight loss and states of malnutrition have been shown to reduce platelet volume and levels [81,85].

Despite the aforementioned findings of increased leukocyte and platelet numbers, these metabolic disorders with increased bone marrow adiposity and suppressed HSC proliferation more often lead to leukopenia and reduced levels of erythrocytes [81,86,87]. Although, the role of adipocytes in

bone marrow is not fully elucidated in detail, current evidence suggests a primarily inhibitory role in HSC niches, thus leading to haematopoietic reconstitution. Emerging evidence implies that obesity- and aging-associated changes in hormonal balance (e.g., leptin ↑, growth hormone ↓) mediate at least to some extent these supposedly adverse changes in HSC niches (Figure 10).

Moderate dietary energy restriction, inhibition of adipogenesis, and reduction in adiposity have the opposite effect on HSC niches as these modalities preserve and even increases haematopoietic activity, prevent HSC niche skewing, and improve the maintenance of repopulation capacity, at least among overweight individuals. Furthermore, even without reduced adiposity, exercise training alone has been shown to normalize bone marrow adiposity and HSC niche proliferation by mechanical stimulation of bone marrow adipocytes and growth-hormone secretion (Figure 10) [88].

On the other hand, prolonged intense exercise training induced ROS production might have counteractive effects on HSC by promoting HSC-aging and exhaustion of HSC niches through enhanced mobilization of early forms of leukocytes (Figure 10) [89,90]. In particular, prolonged exercise bouts cause large releases of neutrophils from the bone marrow, thus making it plausible that repeated bouts of prolonged exercise could actually promote the depletion of bone-marrow reserves of mature neutrophils. Subsequently, highly trained individuals undergoing strenuous exercise regimens and individuals suffering from overtraining have been characterised by low levels of circulating leukocytes, especially neutrophils [91]. Although current evidence demonstrates that exercise training increases the formation of ROS, it has been suggested that the net cellular ROS load is actually reduced by upregulated oxidant scavenging systems [92].

As mentioned earlier, bone marrow adiposity and HSC aging are characterised with diminished erythropoiesis predisposing to anaemia, which are frequently observed in elderly and obese people [80,93]. Both rapid weight loss and malnutrition are also common causes of reduced

erythrocyte numbers and anaemia [86], whereas moderate levels of exercise training have primarily erythropoiesis-inducing effects on HSC niches, thus promoting increments in erythrocyte numbers and haemoglobin mass [94].

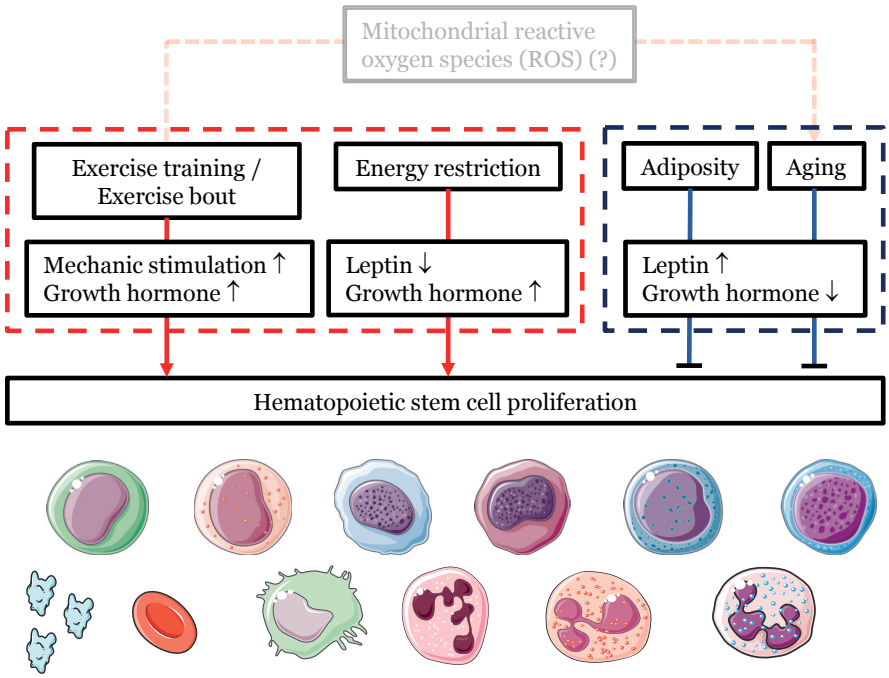


Figure 10. Factors and mediators of haematopoietic stem cell (HSC) proliferation. Arrows (red) indicate positive inducing effects whereas bar-headed lines demonstrate negative inhibitory effects on HSC proliferation. (?) = Current evidence on the association of exercise, exercise-induced reactive oxygen species (ROS) production, and effect on aging is controversial. An illustration website (https://smart.servier.com) was utilized in figure production.

Innate immunity

Exercise training, energy availability, adiposity, and aging also contribute to innate immunity response modulation. Particularly, obesity mediated chronic inflammation has been considered as a link between adiposity, a wide variety of diseases, and innate immune system dysfunction [95]. In excess adiposity, increased activation and levels of innate immune cell

numbers are detected, including neutrophils, monocytes, macrophages, and mast cells.

Although, current findings are somewhat contradictory, it has been demonstrated that obesity, adipose tissue dysfunction, and weight gain have been associated with elevated levels of the total number of leukocytes and especially neutrophils [96,97], and are used as markers for systemic low-grade inflammation. To date, the detailed role of neutrophils in obesity-induced inflammation is incompletely understood, despite the clear association characterised between increased levels of neutrophils and obesity. Moreover, pro-inflammatory cytokines producing classical monocytes and NK cells, and non-classical monocytes are also augmented in numbers in individuals with excess adiposity, where elevated levels of classical monocytes have been shown to reflect macrophage (M1) numbers in visceral adipose tissue [98,99].

In addition, accumulation and augmented activity of mast cells is observed in obesity and chronic diseases [31,100]. Findings from previous studies imply that mast cells may be important in allowing adipose tissue to dynamically expand in response to energy surplus and during weight loss. In adipose tissue of obese humans and animals, mast cell partners, eosinophils and augmented secretion of eotaxin have been detected, thus suggesting an inflammatory activation of the allergic innate immune response [101]. This is a process also potentially contributing to the found link between obesity and increased prevalence of asthma [102]. Early animal models suggested that eosinophils may help to preserve normal metabolic regulation and adipose tissue homeostasis, however, to date the underlying mechanisms remain elusive [103]. Evidence from animal models have also documented an increased number of adipose tissue eosinophils together with reduced macrophage content and inflammatory expression following weight loss [104]. Conversely, it has been proposed that strenuous exercise may be able to cause non-allergic activation of eosinophils [105].

In the past, weight loss has been shown to attenuate levels of overall leukocytes and neutrophils in overweight individuals [106]. Similarly, low body weight in anorexia nervosa has been associated with attenuated levels of neutrophils, despite being characterised as a pro-inflammatory state similar to obesity [81]. Attenuated levels of neutrophils have also been reported in individuals engaging in endurance sports and undertaking intense exercise regimens [91]. Although long-term intensive exercise training has been shown to suppress neutrophil levels, it has been documented that acute exercise bouts have the ability to mobilize neutrophils from bone marrow, thus resulting in increased levels of neutrophils immediately after exercise [107–109].

Adaptive immunity

Exercise training, energy availability, adiposity, and aging also contribute to the modulation of adaptive immunity responses. Dysregulated adaptive immunity leading to immunodeficiency has been observed particularly in severe dietary restriction and the aging immune system, where the effects extend from the structures of peripheral lymphoid organs to adaptive immune-cell proliferation and functions [80,110–112]. Subsequently, malnutrition, nutritional deprivation, and low energy availability have been associated with the atrophy of peripheral lymphoid tissues, dysregulated T-cell proliferation, reduced T-cell maturation, and an imbalance in the ratio of CD4/CD8 T cells, and predominant CD4 type 2 T_H cell response (Figure 11). Following substantial weight loss among overweight individuals, similar fall in T_H1/T_H2 -ratio has also been detected [113], although, more evidence is needed on whether the CD4/CD8 T cell ratio is affected [114].

Moreover, exercise bouts alone have been shown to increase absolute levels of lymphocytes, especially NK cells, thus resulting in the decrease of CD4/CD8 T cell -ratio immediately during and after exercise [115]. Following exercise bouts, however, lymphocyte levels decrease reaching even lower levels than proceeding exercise bouts. Drop in lymphocyte numbers has

been proposed to be caused by increased apoptosis of lymphocytes. In the future, parameters such as volume, intensity, and duration need to be considered when evaluating exercise training effects on adaptive immunity. It has been shown that moderate intensity exercise training guides the immune response towards a predominance of T_H1 cells, while excessive high-intensity exercise training causes increases in the concentrations of anti-inflammatory cytokines mediated by T_H2 responses [116–118].

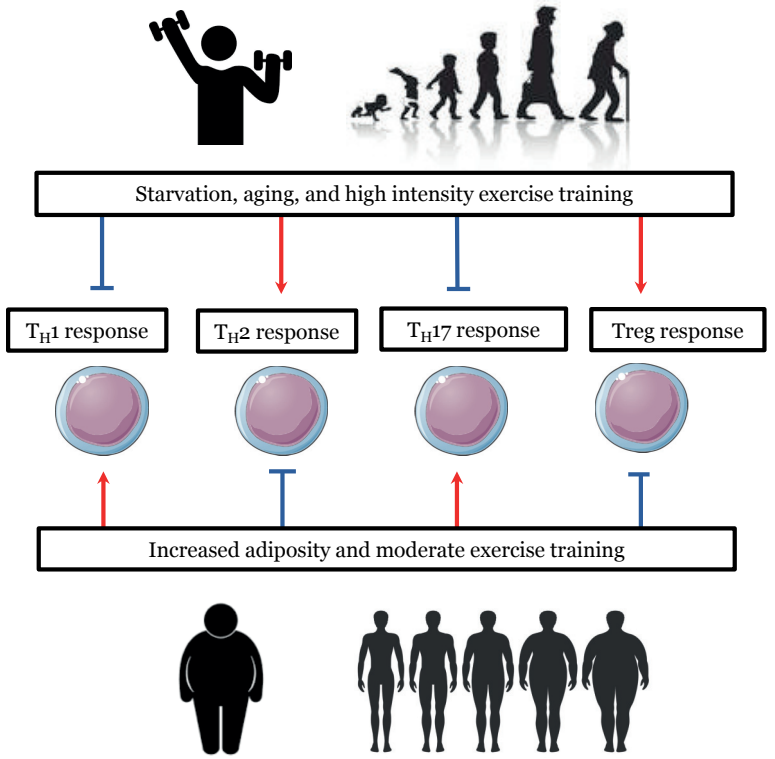


Figure 11. Overview of modulation of T-cell mediated adaptive immune responses by lifestyle factors. Colour of arrows (induction) and bar-headed lines (inhibition) indicate the direction in which factors modulate T cell subset responses. Red = Induction. Blue = Inhibition. T_H = T helper cell. Treg = T regulatory cell. An illustration website (https://smart.servier.com) was utilized in figure production.

It has been implied that both starvation and HSC aging arrest B cell development, diminish the pool of naive B cells, and expand the GC cell pool of memory B cells [119], although, these alterations in B-cell mediated

immunity need to elucidated in more detail, especially in humans. Concomitant with these observations, reduced diversity of the B cell repertoire, lowered antibody affinity, and impaired class switching have also been suggested [80]. Furthermore, B cells are indicated to be more prone to produce auto-antibodies, thus increasing the possibility for increased incidence of spontaneous autoimmunity. Conversely, it has been indicated that when applied in moderation, exercise training and subtle energy restriction might also be potent tools for preventing these adaptive immune system changes associated with starvation and HSC aging [120].

Recent studies have demonstrated adverse modulation of the adaptive immune system in obesity and associated metabolic diseases [121–123]. In these individuals, opposite to the changes observed in dietary restriction and lean phenotype, induced T_H1 and T_H17 helper cell function, inhibition of Treg, increase in CD8 cytotoxic T cells, promoted cytotoxicity of NK cells, and expanded pool of B cells secreting IgGs and proinflammatory cytokines have been reported (Figure 11). However, more studies are needed to elucidate some incoherent findings detected between obese, morbidly obese, and healthy obese individuals [114]. Dysregulation of the adaptive immune system in both energy deprivation and energy surplus have been previously suggested to be mediated by leptin levels as leptin administration or suppression has repeatedly normalized adaptive immune responses [119,124,125].

2.2.2 Cardiometabolic signatures

Detailed high-throughput metabolite profiling studies have demonstrated distinct molecular signatures of obesity, particularly regarding lipids, amino acids, and inflammation biomarker levels [126]. Although weight loss interventions have been shown to mediate favourable effects on these molecular signatures, alleviated levels of cardiovascular risk factors, and future risk of disease, these studies have been predominantly conducted on overweight and obese individuals. To date, a limited number of studies have

investigated whether these molecular signatures of weight change extend across all weight categories, also to individuals with normal-to-low body weight. Independent of weight change, exercise training, physical activity, and healthy diet also have the potential to modulate cardiometabolic signatures in a beneficial manner [127–130].

Inflammation

Obesity-related adverse health outcomes have already been described here as being characterised by low-grade systemic inflammation, whereas dietary restriction leading to weight loss has attenuated the chronic inflammatory response (Figure 12) [131]. However, emerging evidence suggests that the manner of how weight loss is achieved has a distinct impact on the levels of inflammatory mediators as disease-related weight loss (e.g., anorexia nervosa, cachexia) is also characterised by increased levels of proinflammatory cytokines and markers [132]. In addition, chronic pro-inflammatory status is also a pervasive feature in aging, suggested to be mediated by cell senescence and dysregulation of innate immunity [133]. Especially aging-associated increments in inflammation status have been considered to promote the loss of muscle mass and strength (i.e., sarcopaenia), thus underlining a possible existing link between inflammation status and lean-mass compartment [134]. Independent of weight loss, healthy diet and exercise training have both been associated with reduced levels of inflammatory markers (Figure 12) [135,136].

In addition to traditional soluble markers (e.g., cytokines, acute phase proteins) of systemic inflammation, emerging evidence on glycosylation of IgG has provided useful information with regard to inflammation, immune system function, and metabolic health [137]. Glycosylation, the carbohydrate coating of molecules, is known to reflect the physiological state of an organism and its associated changes. As the accumulation of excess adipose tissue has been associated with low-grade

systemic inflammation, assessment of IgG glycosylation provides new tools to quantify inflammation changes in response to weight change.

To date, no intervention studies investigating IgG glycosylation and weight change, lean mass compartments, or exercise training effects has been conducted. However, some studies exist where IgG glycosylation status has been associated with body weight, cardiovascular risk factors, and aging [36,138,139]. Specifically, decreased galactosylation and sialylation, as well as a high degree of fucosylation of IgG Fc, has been characterised as a hallmark of the inflammatory state and poor metabolic health (Figure 12) [36].

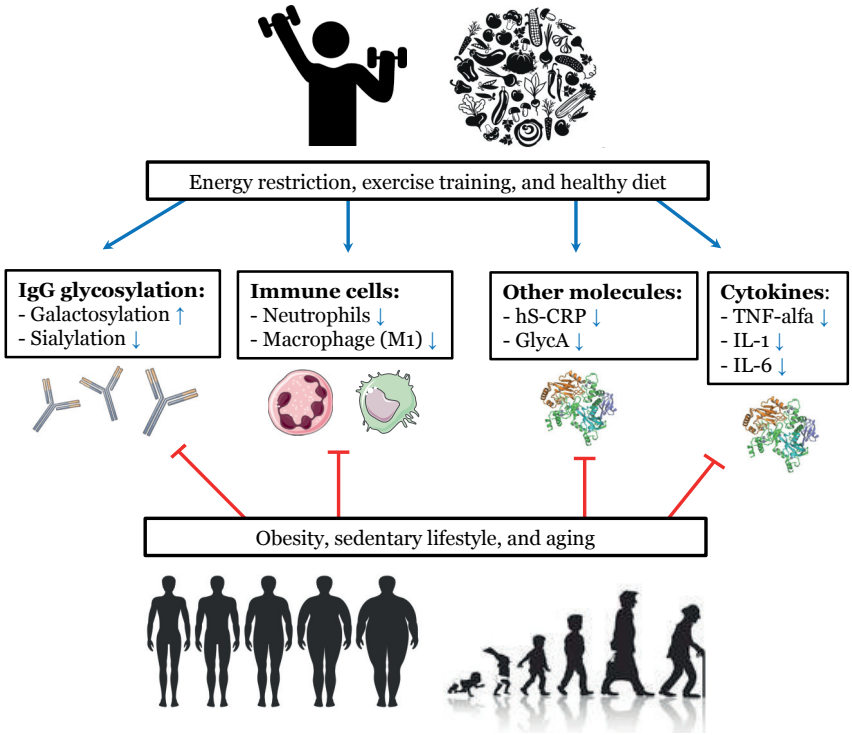


Figure 12. Overview of lifestyle associated factors in the modulation of systemic inflammation mediators. Weight loss, exercise training, and healthy diet modulate the inflammation markers in the direction indicated by the arrows depicted within the boxes. Obesity, sedentary lifestyle, and aging mediate opposite effects (bar-headed lines) on the presented markers of inflammation. IL = interleukin. TNF = tumour necrosis factor. hs-CRP = high-sensitivity C-reactive protein. M1 = classical macrophage. IgG = immunoglobulin G. GlycA = α_1-acid glycoprotein. An illustration website (https://smart.servier.com) was utilized in figure production.

Lipoprotein profile

Of all the physiological entities addressed in this thesis, lipid profile modulation by different lifestyle factors and phenotype characteristics is by far covered in most detail. Altogether, sedentary lifestyle, poor diet, obesity, and aging modulate lipid profiles in an adverse manner, whereas lean body composition, regular exercise training, and healthy diet are associated with an anti-atherogenic lipid profile (Figure 13). Advantageous lipoprotein and lipid profiles have been generally characterised by low levels of total and non-HDL cholesterol together with higher levels of HDL cholesterol [126]. In addition, attenuated levels of TGs and apoB, together with increased levels of apoA1, have also been considered to have anti-atherogenic effects [140,141].

Advancements in metabolomic signature profiling have elucidated that circulatory health is not solely affected by the circulating concentrations of lipoproteins rather than lipoprotein functionality, which is closely connected to composition and particle size. Arising evidence suggests that i) reduced LDL and HDL size together with increased VLDL size, ii) increased TG content of all lipoproteins, iii) increased cholesterol content of non-HDLs, and iv) lower phospholipid content of HDLs are associated with adverse alteration of metabolic health, while beneficial body composition, weight loss, and exercise training have been associated with converse findings (Figure 13) [142–146]. To date, only a limited number of omics studies have been conducted investigating these detailed metabolomic signatures in relation to obesity, weight loss, lean mass, and exercise training.

It has been reported that increased levels of fat mass and weight gain reduce LDL and HDL size while increasing VLDL size, whereas low body weight and weight loss have associated with opposite changes in lipoprotein size [126]. Moreover, regular moderate intensity exercise, both aerobic training and RT, have also been shown to modulate lipid profile in an advantageous manner by increasing HDL-cholesterol levels and preventing the increase of LDL-cholesterol and TG levels [127,147–149]. Recent metabolomic studies

have also discovered that higher cardiorespiratory status and physical activity associate with increased levels of very large-HDL and large-HDL subsets, higher degree of fatty acids unsaturation, and lower levels of VLDL subsets [150,151]. Although, to date, the effects of lean mass alteration on lipoprotein profile and circulatory health is scarcely investigated, some studies have documented associations between lean mass and serum levels of cholesterol and lipid metabolites [152,153].

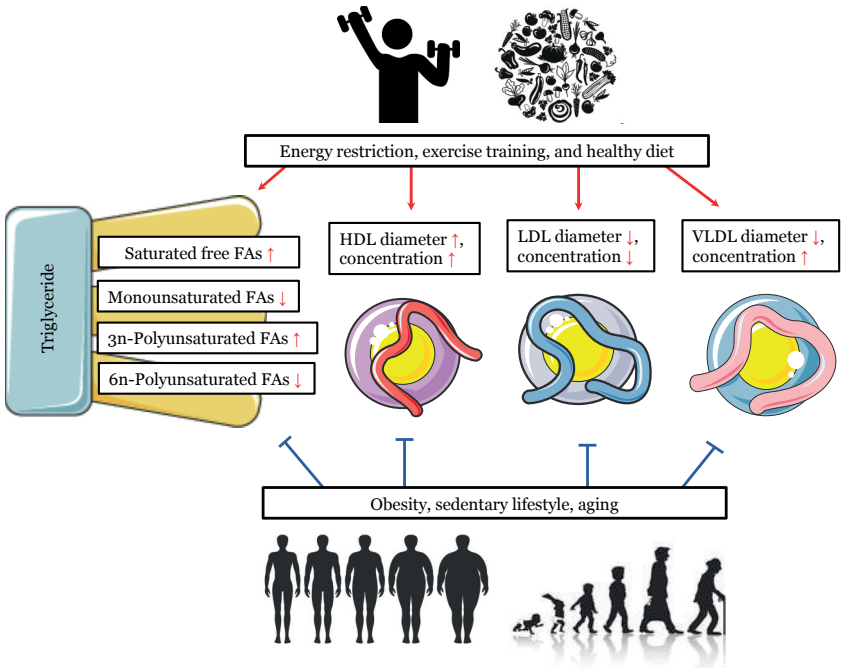


Figure 13. Overview of lifestyle factors affecting lipoprotein and fatty acid profile. Weight loss, exercise training, and healthy diet modulate the inflammation markers in the direction indicated by the arrows depicted within the boxes. Obesity, sedentary lifestyle, and aging mediate opposite effects (bar-headed lines) on the presented markers of inflammation. FA = fatty acid. 3n = omega-3. 6n = omega-6. HDL = high-density lipoprotein. LDL = low-density lipoprotein. VLDL = very low-density lipoprotein. An illustration-website (https://smart.servier.com) was utilized in figure production.

Free fatty acid profile

Elevated total plasma FFAs have been considered detrimental for cardiovascular health and hypothesised to mediate insulin resistance and

the development of T2D, whereas lower levels of circulating FFAs are considered beneficial for metabolic health. To date, evidence on the health effects of different circulating FFA subsets (e.g., SFA, MUFA, PUFA) is somewhat contradictory. Evidence has suggested that increased levels of several FFA subsets (e.g., ω-6 PUFAs, MUFAs, and SFA), to associate with adverse metabolic outcomes [154,155], although, higher levels of ω-6 PUFA have also associated with reduced mortality [156]. Moreover, higher levels of ω-3 PUFAs subsets and conjugated linoleic fatty acids (CLA) have been associated with reduced risk of metabolic disease — even though the emerging evidence is scarce and not self-explanatory [157–160].

In obesity, tissues become saturated with lipids and subsequently result in an increase in FFA export and reduced FFA clearance leading to elevated overall plasma FFAs [161]. Especially, elevated levels of unsaturated FFAs, namely MUFA together with ω-6 PUFA, have been documented in overweight individuals and after weight gain (Figure 13) [154]. Conversely, weight loss has been reported to reduce several circulating fatty acid profile subsets — SFAs, MUFA, and ω-6 PUFAs [154,162]. However, some studies documented findings where lower body weight and weight loss have been associated with increased levels of SFAs and ω-3 PUFAs [154]. It has been suggested that these disparities in findings, especially regarding SFAs, are caused to some extent by differences in length of fatty acids. For example, even-chain SFAs (e.g., palmitic acid / C16:0) have been positively associated, whereas odd-chain and longer-chain SFAs have been inversely associated with incident of diabetes [163].

Exercise bouts are known to simultaneously i) promote insulin sensitivity, and ii) temporarily increase the influx of FFAs to circulation to be used as an energy source. This influx of FFAs immediately following acute exercise impacts FFA profiles by increasing circulating levels of plasma unsaturated FFAs, especially MUFAs [164]. In contrast to these effects of acute exercise bout on FFAs levels, regular exercise training results in attenuated levels of circulating FFAs that is thought to be caused by induced

mitochondrial activity and enhanced FFA oxidation [165]. It has also been hypothesised that reduced FFA levels observed in physically active individuals to be mediated by adipose-tissue remodelling and associated weight loss [155,166]. To date, the available evidence is inconclusive on how exercise training affects *plasma* FFA composition. However, previous studies investigating exercise training effect on *adipose tissue* FFA profiles have provided additional insight into how *adipose tissue* and *plasma* FFA profiles potentially intertwine together. Subsequently, exercise training has been shown to increase the SFA and PUFA, and decrease MUFA content in *adipose tissue* [167] — similar modulations as those observed in the weight loss studies reviewed above.

Amino acid profile

Emerging evidence from metabolic profiling studies has revealed elevated circulating levels of amino acids as prognostic markers of adverse health outcomes, where altered levels are thought to be caused by the metabolic shift resulting from insulin resistance [168–174]. Particularly, augmented levels of branched-chain amino acids (e.g., leucine, isoleucine, valine), aromatic amino acids (e.g., tyrosine, phenylalanine), and alanine have been linked with obesity, and consequently insulin resistance, CVD, and T2D [168–174].

Weight loss, exercise training, and diet interventions have been shown to mostly normalize augmented levels of amino acids and to cause beneficial alterations in circulating amino acid profiles [175,176]. Specifically, low body weight alone has been associated with lower levels of branched-chain amino acids (e.g., leucine, isoleucine, valine), aromatic amino acids (e.g., tyrosine, phenylalanine), and alanine, together with increased levels of glycine and glutamine [126]. Similar to reduced adiposity, long-term physical activity has been shown to potentially reduce circulating levels of aromatic amino acids [150]. Moreover, it has been documented that lean mass associates positively with circulating levels of branched-chain amino acids, tyrosine, and inversely with glycine [174]. Circulating levels of amino acids are

also modulated by dietary protein content independent of weight loss, as diets with average protein content compared to high protein content have greater impact in decreasing circulating concentrations of amino acids [177].

3 AIMS OF THE THESIS

Overall, the present thesis aimed to determine how changes in body composition, exercise training, and energy availability modulate systems biology of complex blood-derived biological networks. Blood was adopted as the choice of tissue as i) blood samples can be attained through minimally invasive measures; ii) blood is the system that transports materials throughout the body and between different tissues involved in exercise and diet, and therefore is an intermediary between relevant tissues; and iii) blood-derived systems biology markers (e.g., leukocyte transcriptomics) have been shown to reflect changes in physiology on a global scale

Specifically, following biological entities were evaluated:

1) **The Female Physique Athlete Study:**
   -The effects of substantial weight loss and subsequent weight regain on serum metabolomics, leukocyte transcriptomics, IgG glycomics, cytokine profile, blood cell proliferation and distribution, and lipid metabolism regulating factors in sample of healthy normal-weight females. The differences in metabolomic profile and its changes between individuals with years of training experience and a healthy lifestyle (i.e., physique athletes), and females from a general population replication cohort of similar age and BMI.

2) **The Male Resistance Training Study:**
   -The effects of short-term RT and associated body composition changes during *ad libitum* diet on the metabolomic markers of normal-weight healthy males.

Systems biology (i.e., omics) studies of this thesis were based on a hypothesis-free approach, where no specific hypotheses were set beforehand. However, it can be argued that all biological research is hypothesis-driven (regardless of dataset scale) even though the hypothesis might be implicit at first. Systems biology hypothesis-free approach broadens the perspective and unveils new molecular details on a global scale, thus potentially revealing a plethora of unknown biological mechanisms. As a limitation, lack of hypothesis in global systems biology research may make it harder to distinguish meaningful biological relationships in complex datasets. To efficiently extract biological meaning from the systems biology datasets i) computational models, ii) explicit, hypothesised relationships, and iii) data simplification can help in distinguishing biologically meaningful traits.

4 MATERIALS AND METHODS

4.1 Study samples and characteristics

4.1.1 The Female Physique Athlete Study

The Fitness/Physique Athlete Study sample consisted of young normal-weight (age: 27.5 ± 4.0 years, BMI: 23.4 ± 1.7 kg/m²) female physique athletes, that volunteered to participate in the present weight loss and weight regain (i.e., weight cycling) study (Figure 14). Initially, the study sample included a total of 60 amateur physique athletes of Caucasian origin competing or aiming to compete in national "Bikini" or "Body fitness" sports who were matched on weight, age, height, and reported training experience (Figure 14). Study participants volunteered into the control group (n=30) or the diet group (n=30), where athletes in both groups had an average of ~3 years of training experience. Participant exclusion criteria for the study were defined as following: i) prevalent diagnosed chronic disease, ii) prescribed medication (e.g., thyroxine) excluding contraception, or iii) individuals with less than 2 years of RT experience.

The participating athletes in both groups were measured at three time points: i) baseline prior to the weight loss regimen (**PRE**), ii) after the diet period of 21.1 ± 3.1 weeks (**MID**), and iii) after the weight-regain period of 18.4 ± 2.9 weeks (**POST**). Volunteered participants in the diet group were engaged in rigorous exercise training and lowered energy intake ensuing weight loss before the competition (**PRE-MID)**, after which they voluntarily recovered to normal levels of body weight and fat by increasing energy intake and reducing the level of exercise during the weight-regain period (**MID-POST)**. In contrast, participants in the control group were instructed to maintain their typical weight and usual fitness lifestyle, including regular exercise training and regular diet, together with trying to maintain aesthetic body fat levels while increasing or maintaining muscle mass throughout the study period (Figure 14). At the three study time points, participants in both groups went through a series of measures, including both anthropometric and physical performance tests. Data

derived from the Female Physique Athlete Study sample was used to generate two publications for the current thesis.

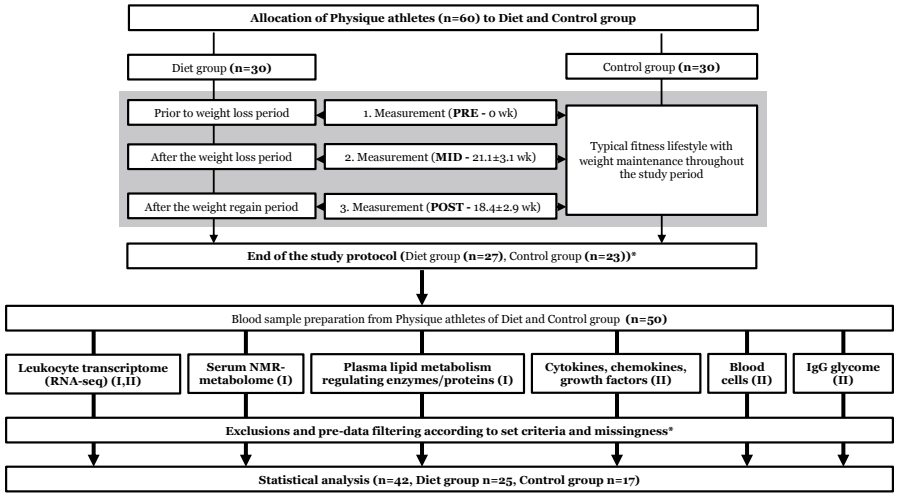


Figure 14. Flowchart of The Bikini Fitness Study design and workflow. *The study began with 60 participants from which 10 failed to complete the designated study regimen, whereas one control did not arrive for baseline testing. In addition, three dieters and six controls were excluded because i) the duration of their weight-regain period was shorter than the other participants, or ii) they failed to completely follow the given instructions. Due to the high cost of large-scale dataset quantification, additional participants that lacked complete dietary records (n = 8) were excluded from the omics study, thus resulting in a total study sample of n=42 volunteers. Furthermore, slight variation in sample size between different downstream analyses was evident due to incompleteness/missingness of omics or phenotype data. In contrast to other measured omics, for the cytokine profile quantification, samples from only 30 individuals were analysed due to high-cost of the analysis panel and limited amount of resources. Designation of systems biology datasets for the Female Physique Athlete Study papers I and II are indicated with roman numerals in the flowchart.

4.1.2 The DILGOM 2007 and 2014 Studies

Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) — DILGOM 2007 and follow-up DILGOM 2014 studies — were utilized as a replication cohort in the Female Physique Athlete Study (paper I) (Figure 15) [178]. The original DILGOM 2007 study was a sub-cohort of the National FINRISK 2007 Study — a national health examination study that was carried out by Finnish Institute for Health and

Welfare (THL) in Finland [17]. FINRISK Studies were conducted every five years to monitor risk factors for major non-communicable diseases in a random sample across five regions in Finland [17]. The DILGOM study aimed to observe more closely how diet, psychosocial factors, lifestyle, environment, and genetics are linked to obesity and metabolic syndrome.

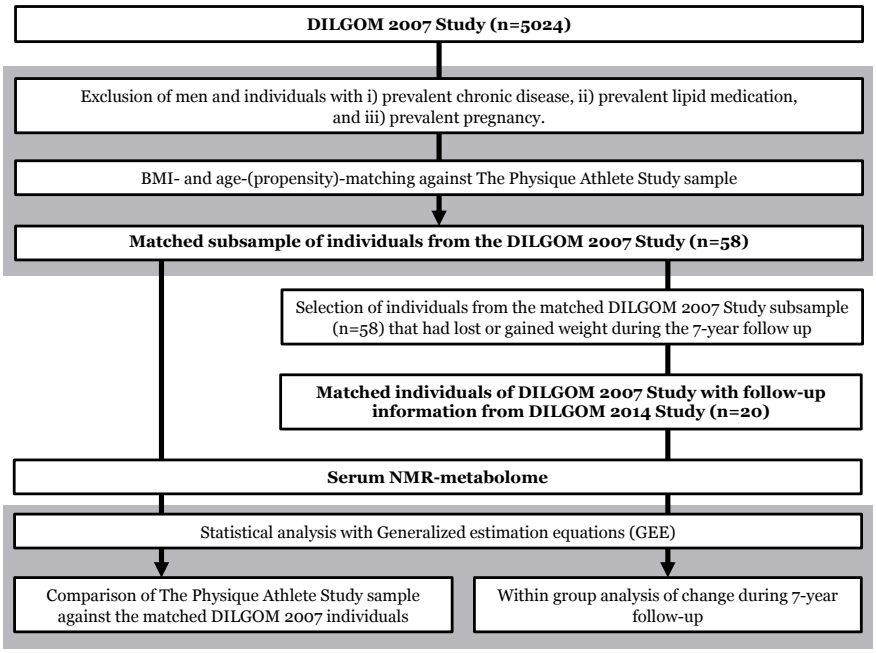


Figure 15. Flowchart of the replication study sample generation and analysis. Study and analysis flow regarding the replication cohort (DILGOM 2007 and 2014 Studies) has been characterized step-by-step in the figure. BMI = body mass index. NMR = nuclear magnetic resonance.

The original DILGOM 2007 study sample (n = 5024) consisted of men and women aged 24–74 years at baseline. From this sample of DILGOM 2007 study individuals, a subsample of BMI- and age-(propensity)-matched individuals (age: 29.3 ± 2.5 years, BMI: 22.9 ± 2.6 kg/m^2) (n = 58) were included in the Female Physique Athlete Study (paper I) of this thesis for the replication of metabolomic findings (Figure 15). Of this subsample (n = 58), information from a 7-year follow-up, between the DILGOM 2007 and

2014 studies, was available for a smaller subset (n = 20), allowing the exploration of how long-term weight loss and weight gain alters the metabolic profile in this subset of young, normal-weight individuals from the general population. In order to further ensure the comparability with the sample of physique athletes, several exclusion criteria were determined: i) prevalent chronic disease, ii) prevalent lipid medication, and iii) prevalent pregnancy.

4.1.3 The Male Resistance Training Study

In the Male Resistance Training Study, total of 86 recreationally active healthy men without previous systematic RT background were included in the research group. Of these individuals, 68 men (age 33 ± 7 years, BMI 28 ± 3 kg/m2) belonged to the RT group and 18 non-training peers (age 31 ± 4 years, BMI 27 ± 3 kg/m^2) belonged to the non-RT group, thus enabling the study of differences in serum metabolome with and without RT (Figure 16). Following study completion, study participants were further divided to high- and low-responders that were defined as the highest and lowest quartile based on the change in lean mass index from the RT intervention. No other variables (e.g., strength) were used to define the responder status. The detailed participant characteristics and methods for the Male Resistance Training Study of this thesis have been reported more extensively elsewhere (RT-group [18,21], non-RT group [19,179]).

The duration of the fully supervised RT intervention was 16 weeks. For RT-group individuals, measurements were performed prior to (**PRE**), after 4 weeks (**POST-4wk**), and after 16 weeks (**POST-16wk**) of RT. The intervention for the RT group individuals began with 4 weeks of whole-body workouts twice-a-week. The participants performed 8–10 exercises within one workout, 2–3 sets for every exercise, and 10–15 repetitions in every set. Recovery time of two minutes was held constant between sets in these workouts. Training loads were 50–80% of one

repetition maximum (1RM) while increasing throughout this preparatory phase of whole-body workouts.

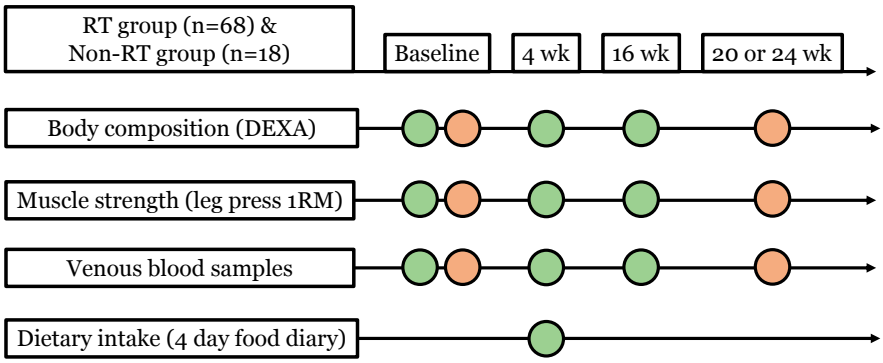


Figure 16. Flowchart of the Male Resistance Training Study design. The duration of the fully supervised resistance-training intervention for the resistance training (RT) group was 16 weeks. Measurements were performed at baseline (PRE), after 4 weeks (POST-4wk), and after 16 weeks (POST-16wk) or resistance training. The non-RT group was measured at baseline (PRE) and after (POST-control) 20-weeks (n=8) or 24-weeks (n=10) into the study periods. The non-RT group was formed using data from two previously collected cohorts which is why the follow-up times vary between groups. Green dots represent RT group, while orange dots depict non-RT group. DEXA = Dual-energy X-ray absorptiometry. 1RM = one repetition maximum.

Nine participants dropped out of the study after 4 weeks of the preparatory RT phase, while the remaining participants (n = 59) were randomized after the first 4-week training period into two groups: i) training aiming for muscle hypertrophy and strength (n = 33) and ii) training aiming for muscle hypertrophy, strength and power (n = 26) for the remaining 12 weeks of the study protocol. The specific RT programs consisted of 2–3 training sessions per week and were divided into three 4-week training blocks in which the volume of hypertrophic (75%–85% loads of 1RM), maximal-strength (86%–95% 1RM), and power-strength (50%–80% 1RM) training fluctuated non-linearly according to the training goal.

The non-RT group was measured before (**PRE**) and after (**POST**) 20-weeks (n = 8) or 24-weeks (n = 10) into the study period, where follow-

up intervals differed since the non-RT group was formed using data from two previously collected cohorts.

4.1.4 Ethics declaration

All of the studies were conducted according to the Declaration of Helsinki with the participants giving written informed consent. The study samples have been reviewed and approved by their respective ethical committees: i) the Ethics Committee at the University of Jyväskylä (the Female Physique Athlete Study, the Male Resistance Training Study), ii) the Ethics Committee of the Central Hospital, Jyväskylä (the Male Resistance Training Study), and iii) the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District (DILGOM 2007 and 2014 Studies).

4.2 Phenotype screening methods

4.2.1 Anthropometric measures

In both the Female Physique Athlete Study and the Male Resistance Training Study, body composition and anthropometrics (including total fat mass, lean mass, and visceral fat mass) were assessed primarily with Dual-energy X-ray absorptiometry (DEXA, Lunar Prodigy Advance, GE Medical Systems – Lunar, Madison WI USA). Visceral fat mass estimates were derived from automatic analyses calculated from the android region (i.e., the area between the ribs and the pelvis) [180]. For purposes of the Male Resistance Training Study, measured total body lean and fat mass were normalized to body surface area (BSA). BSA was calculated using the following formula: BSA (m^2) = square root of (height (cm) x weight (kg)/3600) [181]. In addition to DEXA, B-mode axial plane ultrasound (2D US, model SSD-α10, Aloka, Tokyo, Japan) was used to estimate arm (triceps brachii) and leg (vastus lateralis) subcutaneous fat thickness in the Female Physique Athlete Study. Waist circumference and BMI were determined with conventional methods in all studies of this thesis. Body mass index was calculated as measured weight in kilograms divided by squared height in

meters (kg/m^2), whereas waist circumference was measured midway between the lower rib margin and iliac crest with rounding to the nearest 0.5 cm.

4.2.2 Physical activity and muscular fitness

In the Female Physique Athlete Study, total physical activity level is reported in a similar manner for the Physique study participants and DILGOM 2007 replication cohort using metabolic equivalent hours per week (METh/wk). Physique study participants reported i) type, ii) duration, and iii) intensity of daily physical activity throughout the study (PRE, MID, POST) from which overall physical activity (METh/wk) was calculated. Overall physical activity level for the DILGOM 2007 Study individuals was determined by deriving from the short form of International Physical Activity Questionnaire (IPAQ) reported during the DILGOM 2007 Study 2007 collection [182]. From the IPAQ derived answers, the level of physical activity (METh/wk) was calculated using predefined IPAQ scoring protocol (https://sites.google.com/site/theipaq/home). No information was available on physical activity from the DILGOM Study 2014.

In the Male Resistance Training Study, muscular fitness was assessed with maximal bilateral dynamic concentric strength of the leg extensors (hip and knee extensors) that was measured using a horizontal leg press device (D210, David Health Solutions Ltd., Helsinki, Finland). The highest load (kg) that the participants were able to lift to a full knee extension (180°) was accepted as the 1RM.

4.2.3 Dietary intake

The Female Physique Athlete Study athletes reported dietary intakes repeatedly with food diary entries on representative days throughout the study. The diet group individuals reported intakes at baseline (PRE), after the weight-loss period (MID), and after the weight-regain period (POST) (Figure 14). The control group participants of the Female Physique Athlete Study reported dietary intakes at similar time intervals throughout the

study period (PRE, MID, POST). In the Male Resistance Training Study, dietary information was gathered at the end of the first 4-week preparatory training period using four-day food diary (PRE-4wk) (Figure 16). The subjects also received both verbal and written nutritional recommendations based on the dietary guidelines for normal healthy adults. Participants were instructed to maintain their dietary behaviour and intake constant throughout the study period. The food diaries provided by the participants in all studies of this thesis were analysed by dietary analysis software (Aivodiet, Flow-team Oy, Oulu, Finland).

For the DILGOM cohort, dietary information was collected using the Food Frequency Questionnaire (FFQ) and the dietary information was calculated by using the national food composition database (Fineli) and an in-house software (Finessi) [17].

4.3 High-throughput systems biology screening methods

4.3.1 Blood sample collection

For all study samples investigated in this thesis, fasting serum samples were always collected at the same time of day after at least eight hours of fasting. Within each study setting, repeatedly collected blood samples were attained in a similar fashion to minimize possible bias in sample collection. The designation of the quantified systems biology dataset in the Female Physique Athlete Study (papers I and II) of this thesis was illustrated in Figure 14. In the Male Resistance Training Study and DILGOM 2007 and 2014 Studies, only serum metabolomics were quantified. All blood sample preparation, processing, and quantification of systems biology markers were performed by professional laboratory personnel.

4.3.2 Metabolomics

The same high-throughput serum Nuclear Magnetic Resonance (NMR) metabolomics platform (Nightingale Health Ltd, Helsinki, Finland) was

used in all studies of this thesis for the absolute quantification of serum metabolites [183]. The collected serum samples were measured by utilizing a Bruker AVANCE III HD NMR 500 MHz spectrometer equipped with a cryogenically cooled TCI CryoProbe Prodigy, where the used measurement temperature was 310.1 K. The full process and methods of sample preparation and quantification have been described elsewhere [183]. In all studies of this thesis, the NMR metabolome platform yielded a total of 228 different metabolites, including an array of lipoprotein subclasses (e.g., VLDL, LDL, IDL, HDL), apolipoproteins, serum FFAs, and a wide variety of small molecules such as glycolysis precursors, amino acids, and inflammation biomarkers.

4.3.3 Transcriptomics

Library preparation

The sequencing RNA library of each provided sample was prepared utilizing Illumina TruSeq according to the protocol provided by the manufacturer (www.illumina.com). The utilized Illumina protocol was specified as i) paired-end, ii) strand-specific, and iii) the applied read depth for library preparation was set to 2 x 100 base-pairs. All >200 base-pair RNA was included in the prepared RNA-libraries regardless of PolyA-tail content. Ribosomal RNA was excluded from leukocyte samples accordingly with the utilized ribodepletion method. Following RNA-library preparation, sequencing of the RNA libraries was performed utilizing the Illumina HiSeq2000 sequencing platform.

4.3.4 Glycomics

IgG isolation, glycan release, and labelling

The entire procedure necessary was performed as previously reported in more detail by Pučić et al. [184]. At first, IgGs were isolated from plasma samples by utilizing Protein G 96-well plates (BIA Separations, Slovenia). The isolated IgGs were denatured with the addition of Sodium Dodecyl

Sulphate (SDS) (Invitrogen, USA) and by introducing incubation at 65°C, while the excess SDS was neutralized by using Igepal-CA630 (Sigma-Aldrich, USA). Furthermore, N-glycans were released following the introduction of PNGase F (Promega, USA) in Phosphate Buffered Saline, where the released N-glycans were labelled with 2-AB. Free label and reducing agents were extracted from the samples by utilizing hydrophilic interaction liquid chromatography solid-phase extraction (HILIC-SPE). In the end, glycans were eluted with ultrapure water and stored at –20°C until further utilization.

Ultra-performance liquid chromatography (UPLC)

Fluorescently labeled N-glycans were separated by HILIC on the Acquity UPLC instrument (Waters, USA) consisting of a quaternary solvent manager, sample manager, and an FLR fluorescence detector set, while the excitation and emission wavelengths were set at 250 and 428 nm. The instrument was controlled by Empower 3 software, with the build 3471 (Waters, USA). Labeled N-glycans were separated on a Waters BEH Glycan chromatography column, including 100 × 2.1 mm i.d., 1.7 μm BEH particles, with i) 100 mM ammonium formate (pH 4.4) as solvent A, and ii) acetonitrile as solvent B. The separation method utilized a linear gradient of 25 – 38% solvent A at flow rate of 0.40 ml/min in a 27 min analytical run. Analysed samples were maintained at the temperature of 10 °C before injection, while the separation temperature was 60 °C. Data processing was performed utilizing an automatic procedure with a traditional integration algorithm, after which each generated chromatogram was manually corrected to maintain the same intervals of integration for all the investigated samples. All obtained chromatograms were separated in the same manner to generate 24 peaks containing distinctive N-glycans [182]. The amount of glycans in each of these generated 24 peaks were expressed as % of total integrated area. Derived traits were calculated according to the following formulas: i) for agalactosylated G0 = GP1 + GP2 + GP3 + GP4 + GP6; ii) with one galactose G1 = GP7 + GP8 + GP9 + GP10 + GP11; iii) with

two galactoses G2 = GP12 + GP13 + GP14 + GP15; and iv) sialylated glycans S = GP16 + GP17 + GP18 + GP19 + GP21 + GP22 + GP23 + GP24.

4.3.5 Cytokines

Cytokine quantification with Multiplexed Luminex analyses

Serum concentrations of designated cytokines, chemokines, and growth factors were determined using the 38-plexed Milliplex MAP Kit (cat.no. HCYTMAG-60K-PX38) according to the protocol provided by the manufacturer (Merck-Millipore Corp., Billerica, MA, USA). Quantification of the aforementioned markers was performed by utilizing a Bio-plex 200 Luminex-instrument and Bio-Plex Manager software (Bio-Rad, Sweden). Concentration of each measured marker was determined from an 8-point standard curve using five parameter logistic regression. Minimum detectable concentration (MinDC) was set for each marker separately with the lowest concentration observed on the standard curves linear phase [MinDC=c(low)+2 standard deviation (SD)], where samples below MinDC were designated with a value of 50% of MinDC.

4.3.6 Regulating factors of lipid metabolism

Quantification of enzyme activities and protein contents of lipid metabolism regulators – PLTP, PON1, CEPT, LCAT, ANGPTLs

CETP activities were analysed by using a radiometric method, where transfer/exchange of radiolabelled [14C] cholesteryl oleate (Amersham Biosciences) occurred between exogenously added human LDL and HDL [185,186]. Liquid scintillation counting was employed to determine the radioactivity of HDL as a measure of transfer activity. For the radiometric PLTP activity assay, radiolabelled phosphatidylcholine liposomes were prepared and the activity assay was performed as described previously [187]. Prior to analysis of PLTP, the fasting serum samples were diluted in a ratio of 1:10 with assay buffer, and subsequently 4 µl of the dilution was used for the phospholipid transfer assay. Following incubation, liposomes were

precipitated and the radioactivity in HDL was determined by liquid scintillation counting. LCAT activities were measured using a radiometric method that employed radiolabeled reconstituted apoAI-discoidal particles as substrate [188]. A chromogenic method was used to measure PON-1 activity [189]. For all of these assays determined lipid metabolism markers, intra- and inter-assay coefficient of variation (CV) ranged between 7% to 16%. Plasma levels of ANGPTL3 and 4 were determined using ELISA methods as described previously by Robciuc et al. [190], whereas ANGPTL8 was measured by ELISA method described previously by Tikka et al. [191].

4.3.7 Erythrocytes, platelets, and white blood cells

Differential count analysis of blood cell distribution

Following sample extraction, whole blood samples were analysed within 30 minutes. Total and differential WBCs were measured with Sysmex KX-21N (TOA Medical Electronics Co., Ltd, Kobe, Japan). From the assay, neutrophils, lymphocytes, and mixed cells (monocytes, eosinophils, basophils and immature precursor cells) were separated and quantified.

4.3.8 Other quantified biomarkers

High-sensitivity C-reactive protein

Concentration of high-sensitive CRP (hs-CRP) concentration was determined in the central laboratory of THL during the DILGOM 2007 and 2014 studies, and from the serum samples of Physique study participants. Information of this acute phase biomarker was utilized in the Female Physique Athlete Study (paper I).

4.4 Statistical analysis

4.4.1 Data quality control

The software used for all statistical analysis in this thesis was R (version 3.3.3 or higher) (https://www.r-project.org) and related packages. For all datasets included in this thesis, skewness, normality, and outliers were

assessed with dot plots and histograms to ensure data quality and reducibility of the results. Outliers were removed based on variable SD diversion from the mean depending the variance of quantified measures. At first, extreme outliers based 10SD deviation from was excluded before setting the final exclusion threshold (2-4SD) depending on the variance observed in the dataset. No transformation was applied for generalized estimation equation (GEE) analyses due to the semi-parametric nature of the method.

4.4.2 Statistical methods

GEE – Generalized Estimation Equations

For all repeated measures datasets in the Female Physique Athlete Study and the Male Resistance Training Study (excluding leukocyte transcriptomics), GEE with linear link and working independence correlation structure [192] was used to investigate whether time-dependent changes in measured system biological factors occurred. For all primary analyses, the observations within a single person between time different time points (PRE, MID, POST) were analysed as a matched pair (PRE-MID, PRE-POST). In the analysis, between-subject variability and age were also accounted for. Additional covariates (e.g., BMI, energy intake, physical activity level) were included in models when needed, to reduce statistical noise and determine underlying independent effects. Repeated measures analyses were mainly focused on within-group analysis to ensure result validity since several differences were observed in the baseline values of the diet group and control group within the Female Physique Athlete Study. In within-group analyses, each study participant acts as his/her own control, and hence biases that may arise due to any inexact matching of cases and control groups are avoided in such within-group analyses. Between-group and interaction-modelling (time*group) was applied where necessary to separate true affected system biological factors from false significant findings (e.g., seasonal changes, Hawthorne effect).

DESeq2 - Differential expression analysis of gene level data

For the leukocyte RNAseq data utilized in the Female Physique Athlete Study, sequence alignments were further processed within the R interface by employing the DESeq2 software to assemble transcripts, quantify the expression levels, and analyse differentially expressed genes (DEGs). Genes with very low expression were excluded from the statistical analysis.

For the detection of DEGs, Likelihood ratio test was utilized to conduct a nested time-course study with DESeq2 software (H_0 = Group + Time + Group*Subject, H_1 = Group + Time + Group*Subject + Group*Time) to investigate whether genes were differentially expressed between the diet and control groups across any of the time points when accounting for between-subject variability. To further explore within group changes, post-hoc analysis of the Likelihood ratio test was also performed for the diet and control groups only (H_0= Subject, H_1= Subject + Time). In addition, Wald-tests were applied within the DESeq2 interface for testing contrasts and deriving specific log2FoldChanges and *P* values for the between/within group comparison across any of the individual time points (PRE-MID, PRE-POST).

CPDB - Pathway analysis of gene level data

Downstream pathway analysis was performed to identify i) enriched and ii) over-represented biological pathways. The web-based tool ConsensusPathDB-human (CPDB) integrated database (http://cpdb.molgen.mpg.de) was utilized for pathway analysis of transcriptomic data. Both enrichment and over-representation analyses were focused on to determine significantly affected pathways from the Reactome and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. A pre-ranked list of DEGs was supplied to the database engine to calculate significantly altered pathways. A DEGs list was ranked based on $\log_{10}$(p-value) *sign($\log_2$FoldChange) to account for both, significance of differential expression and magnitude of expression change

between/within groups across time points. Analyses were performed separately for lists of up- and downregulated DEGs to increase power, where only DEGs were employed to exclude redundant genes from the analysis.

4.4.3 Multiple testing correction

In this thesis, all analyses conducted in the Female Physique Athlete Study, the Male Resistance Training Study, and DILGOM 2007 and 2014 Studies were adjusted for multiple testing using the Benjamini-Hochberg procedure (false discovery rate, FDR), where significance threshold was set according to FDR < 0.05 across all datasets. Multiple testing correction using FDR was applied within each omics domain separately but not across different omics categories.

5 RESULTS

5.1 The Female Physique Athlete Study: Systems biology of weight loss and weight regain

5.1.1 Adiposity compartments and anthropometric phenotype

In the study sample of normal-weight female physique athletes (n = 42), after the 20-week weight-loss period the diet group had achieved a significant (FDR < 0.05) reduction in fat mass across all fat-mass compartments as summarized in Table 1. Particularly, the intensive weight-loss period in the diet group resulted in a 73% reduction in visceral fat mass (FDR < 0.05), which contributed to a 52% decrease in total body fat mass (FDR < 0.05), and an 8% reduction in waist circumference (FDR <0.05). Substantial weight loss was accomplished through a combination of 19% decrease in energy intake and 15% increase in total volume of exercise as assessed by relative energy expenditure (METh/wk). Together, the decrease in energy intake and increase total volume of exercise resulted in 29% decrease in energy availability during the weight-loss period (PRE-MID) (Table 1). Despite the prolonged period of low-energy availability, no significant (FDR > 0.05) loss of lean mass was observed concomitant with the substantial weight loss (Table 1).

Closer examination of adipose tissue distribution revealed distinct differences in inter-correlation between upper and lower adipose tissue compartments in the physique athlete sample (n=42) across all time points (PRE, MID, POST). For upper body adipose tissue measures, visceral fat mass was highly correlated with total fat mass (r = 0.94, $P < 2.0 \times 10^{-16}$) and arm adipose tissue thickness (r = 0.64, $P = 3.42 \times 10^{-13}$), but not as strongly with leg adipose tissue thickness (r = 0.26, $P = 2.15 \times 10^{-2}$) representing lower body adiposity. Only modest correlations (r < 0.4) were detected between adiposity, levels of exercise training, and energy intake in the physique athlete sample (n = 42).

Table 1. Charasteristics of body composition, exercise level and dietary intake alterations in the Physique study groups and general population comparison FINRISK Study participants

	Diet group (PRE)	Diet group (MID)	Diet group (POST)	Control group (PRE)	Control group (MID)	Control group (POST)	FINRISK
Weight (kg)	64.7 (6.9)	56.6 (5.5)*	63.2 (6.9)*	63.7 (5.0)	64.0 (5.8)	63.6 (5.6)	62.1 (7.8)
BMI (kg/m^2)	23.5 (1.8)	20.6 (1.4)*	23.0 (2.0)*	23.1 (1.4)	23.2 (1.8)	23.1 (1.8)	22.9 (2.6)
Fat mass (kg)	14.9 (4.5)	7.2 (2.7)*	13.0 (4.2)*	14.2 (3.1)	14.9 (3.5)	14.4 (3.2)	17.8 (5.6)*
Lean mass (kg)	47.7 (4.2)	48.1 (4.0)	48.5 (4.4)*	47.5 (3.8)	47.4 (3.8)	47.5 (4.1)	44.3 (2.9)*
Waist circumference (cm)	75.7 (4.3)	69.6 (3.0)*	74.2 (3.9)*	74.2 (3.5)	74.0 (4.5)	72.9 (4.5)*	76.5 (7.2)
Waist:Hip -ratio	0.8 (0.03)	0.8 (0.04)	0.8 (0.03)	0.8 (0.03)	0.8 (0.03)	0.8 (0.03)*	0.8 (0.1)
Visceral fat mass (g)	937.9 (324.3)	249.6 (144.6)*	840.8 (306.8)	919.4 (327.7)	984.7 (379.4)	902.3 (350.3)	
Leg fat mass thickness (cm)	1.0 (0.3)	0.6 (0.2)*	0.8 (0.3)*	1.0 (0.3)	1.0 (0.3)	1.1 (0.4)*	
Arm fat mass thickness (cm)	0.9 (0.3)	0.7 (0.5)*	0.9 (0.4)	0.8 (0.2)	1.0 (0.2)*	0.9 (0.2)*	
Total exercise level (METh/wk)	59.3 (13.8)	68.4 (19.6)*	53.2 (16.2)	49.4 (27.8)	41.8 (18.7)	48.8 (27.0)	31.3 (19.7)*
Resistance training (METh/wk)	45.3 (8.8)	46.1 (9.9)	42.3 (8.2)	33.6 (19.4)	28.6 (14.2)	32.1 (17.8)	
Aerobic exercise (METh/wk)	13.9 (10.4)	22.3 (17.8)*	11.0 (12.2)	15.7 (23.8)	13.2 (14.5)	16.7 (25.6)	
Energy intake (kCal/kg)	36.5 (6.5)	29.6 (5.5)*	37.8 (9.9)	39.6 (8.0)	36.8 (5.8)	39.7 (5.5)	32.9 (10.2)*
Protein (g/kg)	3.1 (0.6)	3.1 (0.7)	3.3 (0.8)	2.8 (0.5)	2.8 (0.5)	2.9 (0.5)	1.4 (0.5)*
Carbohydrate (g/kg)	3.4 (1.0)	2.1 (0.6)*	3.2 (1.3)	3.6 (0.6)	3.4 (0.6)	3.6 (0.8)	4.0 (1.4)*
Fat (g/kg)	1.0 (0.3)	0.8 (0.2)*	1.0 (0.2)	1.3 (0.4)	1.2 (0.4)*	1.4 (0.5)	1.1 (0.4)
Energy availability (kCal/kg/FFM/day)	36.3 (9.2)	24.6 (7.5)*	28.7 (8.8)*	40.5 (11.5)	38.6 (8.1)	44.8 (9.4)	

METh/wk = metabolic equilevant hours per week. kCal = kiloCalories. FFM = fat free mass. Values are presented as mean (standard deviation, SD). Means and SD's are calculated for the physique athletes n=42. *Statistical significant diffence i) from baseline ($P < 0.05$) within Physique group comparisons and ii) between pooled Physique study participant baseline and FINRISK Study participants. Significance was calculated with Generalized Estimation Equations where age was accounted for in the model. Descriptives for general population comparison, the FINRISK Study cohort, was derived from the age- and BMI-(propensity score)-matched individuals n=58.

5.1.2 Haematopoiesis and bone metabolism

Prolonged low-energy availability and intense exercise leading to reduced overall adiposity resulted in a 1.3% loss of overall bone mass in the diet group as was reported previously for the same study population [16]. Enhanced turn-over from bone tissue was accompanied by potentially altered proliferation and survival of HSCs as was suggested by differential expression (FDR < 0.05) of transcriptional key regulating factors of HSCs and altered distribution of circulating blood cell levels (Figures 17 and 18).

Specifically, substantial weight loss led to increased ($P < 0.05$) levels of total WBCs, that was mostly explained by augmented neutrophil numbers (Figure 17). Furthermore, signs of moderate elevation ($P > 0.05$) in granulocyte (i.e., mixed cell) subsets was detected, thus suggestive of promoted proliferation and lifted quiescence of several white blood cell lines (Figure 17). On the other hand, reduction ($P < 0.05$) in erythrocyte counts was detected together with modest attenuation ($P > 0.05$) in platelet levels, suggestive of suppressed development of the erythroid/megakaryocyte progenitor lineage in bone marrow (Figure 17).

After substantial weight loss, evidence of increased susceptibility to metabolic stress by ROS on the pool of HSCs was suggested through attenuated expression of FOXO transcription factors (e.g., *FOXO4*) that are essential for protecting HSCs from the negative effects of ROS (Figure 18). Furthermore, augmented apoptosis in the pool of HSCs was suggested by distinct suppression of anti-apoptotic transcription factor expression signatures (e.g., *BCL2L1*, *PRDX6*) (Figure 18). No long-term effects were detected on HSC regulating transcription factors or circulating levels of leukocytes, erythrocytes, or platelets as observed changes reverted back to baseline during the subsequent voluntary weight-regain period (Figures 17 and 18).

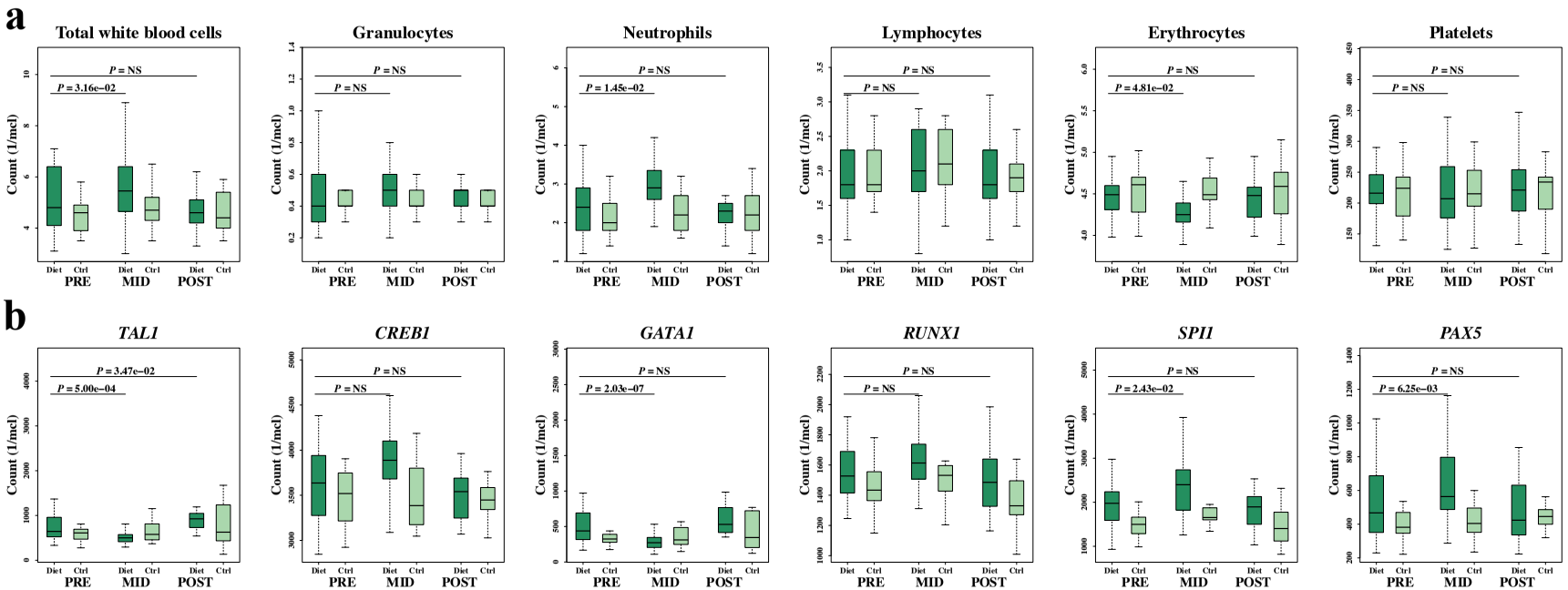
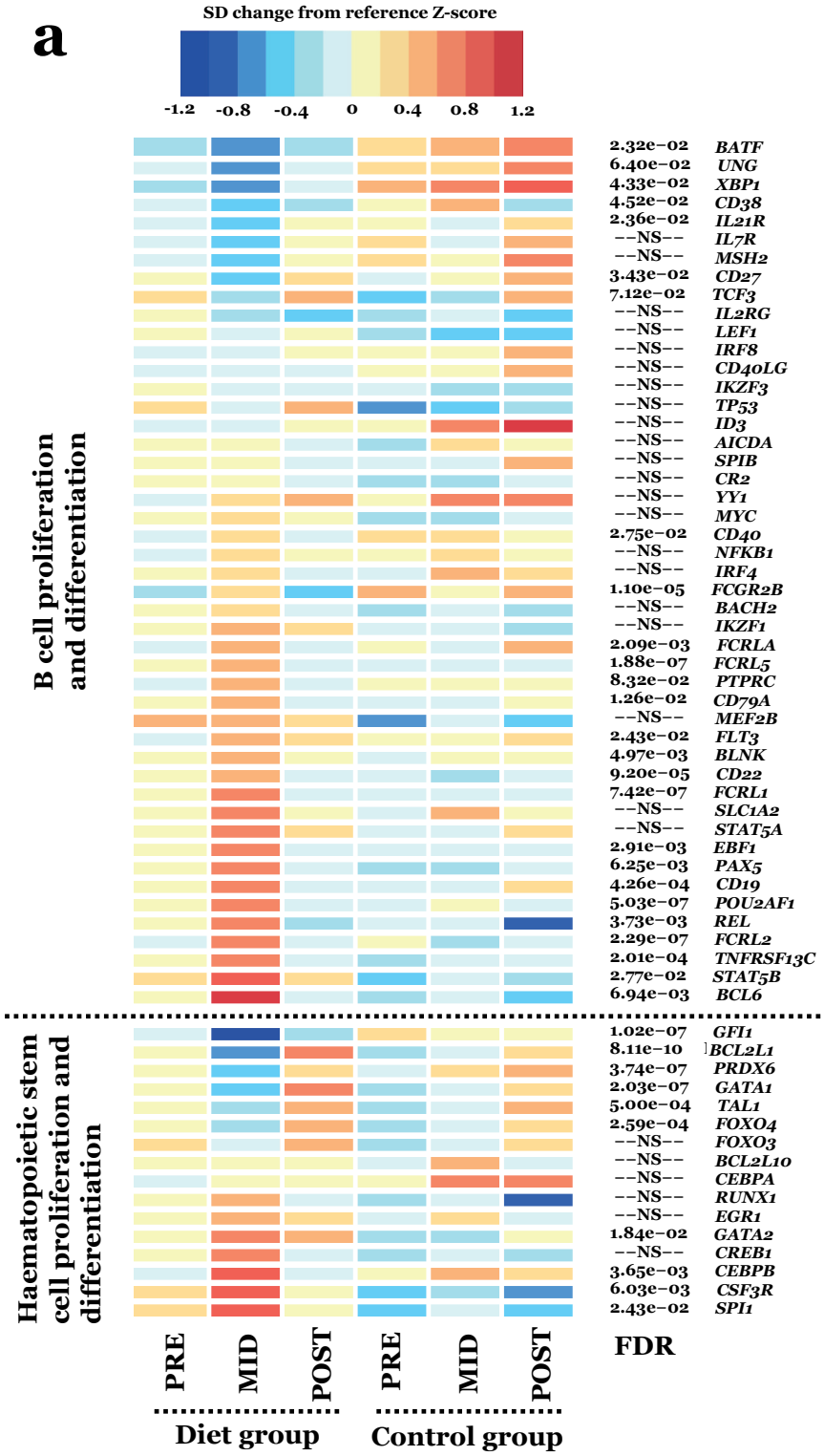


Figure 17. Levels of blood cells and transcription factors participating in the regulation of haematopoiesis. Panel a. depicts the absolute levels of different categories of quantified blood cells whereas Panel b. demonstrates differential expression levels of transcription factors affecting blood cell proliferation (i.e., haematopoiesis). NS = Not significant ($P > 0.05$ on panel a, FDR > 0.05 on panel b). Ctrl = Control group. FDR = false discovery rate. Granulocytes depicted in the figure include basophils, eosinophils, and monocytes (*i.e.,* mixed cells).


a
SD change from reference Z-score
-1.2
-0.8
-0.4
0
0.4
0.8
1.2
B cell proliferation and differentiation
2.32e−02 BATF
6.40e−02 UNG
4.33e−02 XBP1
4.52e−02 CD38
2.36e−02 IL21R
−−NS−− IL7R
−−NS−− MSH2
3.43e−02 CD27
7.12e−02 TCF3
−−NS−− IL2RG
−−NS−− LEF1
−−NS−− IRF8
−−NS−− CD40LG
−−NS−− IKZF3
−−NS−− TP53
−−NS−− ID3
−−NS−− AICDA
−−NS−− SPIB
−−NS−− CR2
−−NS−− YY1
−−NS−− MYC
2.75e−02 CD40
−−NS−− NFKB1
−−NS−− IRF4
1.10e−05 FCGR2B
−−NS−− BACH2
−−NS−− IKZF1
2.09e−03 FCRLA
1.88e−07 FCRL5
8.32e−02 PTPRC
1.26e−02 CD79A
−−NS−− MEF2B
2.43e−02 FLT3
4.97e−03 BLNK
9.20e−05 CD22
7.42e−07 FCRL1
−−NS−− SLC1A2
−−NS−− STAT5A
2.91e−03 EBF1
6.25e−03 PAX5
4.26e−04 CD19
5.03e−07 POU2AF1
3.73e−03 REL
2.29e−07 FCRL2
2.01e−04 TNFRSF13C
2.77e−02 STAT5B
6.94e−03 BCL6
Haematopoietic stem cell proliferation and differentiation
1.02e−07 GFI1
8.11e−10 BCL2L1
3.74e−07 PRDX6
2.03e−07 GATA1
5.00e−04 TAL1
2.59e−04 FOXO4
−−NS−− FOXO3
−−NS−− BCL2L10
−−NS−− CEBPA
−−NS−− RUNX1
−−NS−− EGR1
1.84e−02 GATA2
−−NS−− CREB1
3.65e−03 CEBPB
6.03e−03 CSF3R
2.43e−02 SPI1
FDR
PRE
MID
POST
PRE
MID
POST
Diet group
Control group

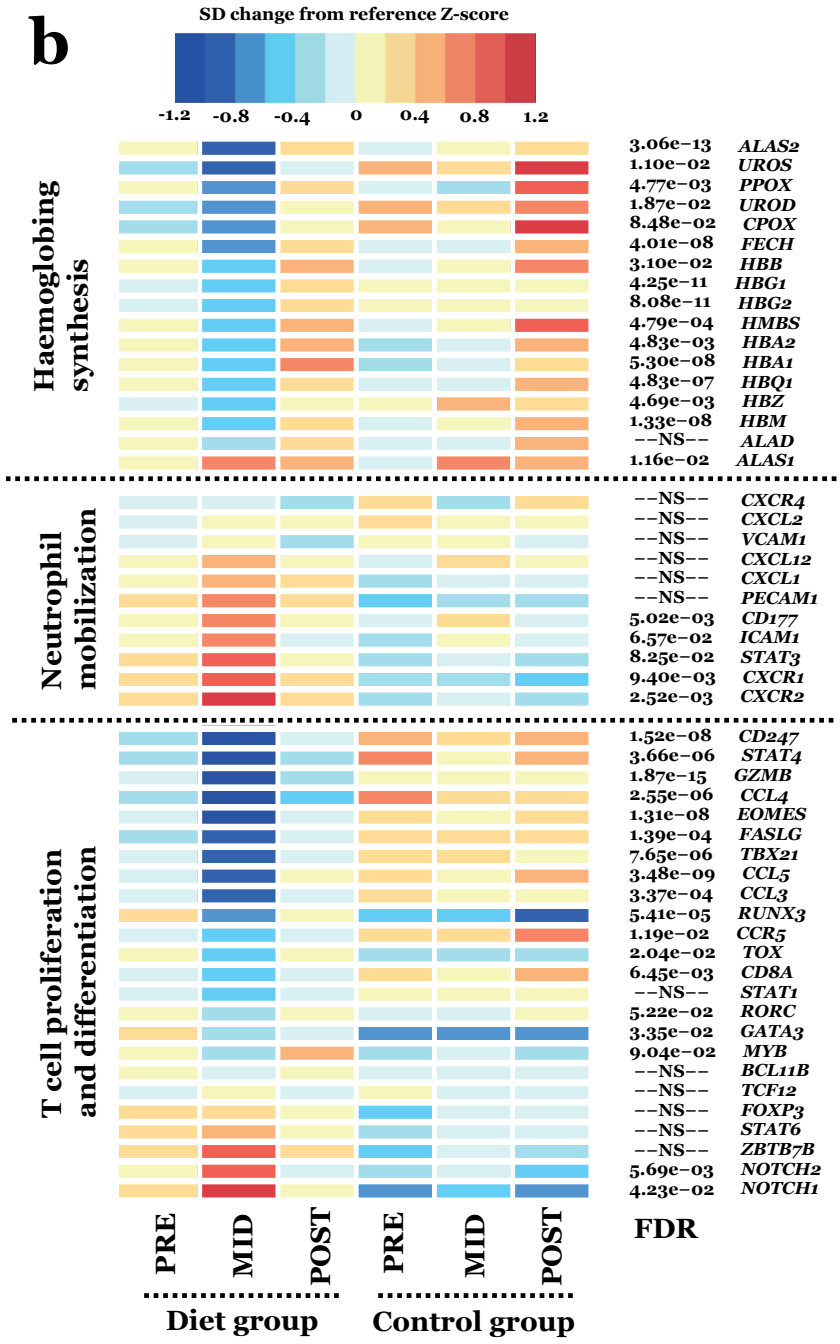


Figure 18. Expression patterns of genes related to the regulation HSC homeostasis and immune system throughout the study period (panels a and b). Heat map is derived from DESeq2 normalized expression levels that are represented as standard deviation (SD) change from the reference Z-score, that was calculated from the pooled baseline Z-score (PRE) of both the diet and control groups. Each individual group/time-point level was compared against the formulated reference value. FDR = false discovery rate of diet group (PRE-MID). FDR > 0.1 was set as not significant (NS). Significance is calculated from Wald test contrast from time-point interval comparison within diet group.

5.1.3 Immune system

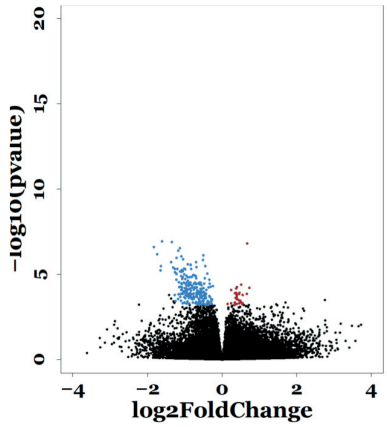
Overall suppression of the immune system was suggested by downregulation (*Q* value < 0.05) of the immune system pathway in the leukocyte transcriptomic analysis (Figure 19). Immune system classification into innate and adaptive immune system is an oversimplification as they intertwine closely. However, for clarity of reporting, specific findings on the immune system in this thesis are reported according to these two categories.

Innate immune system

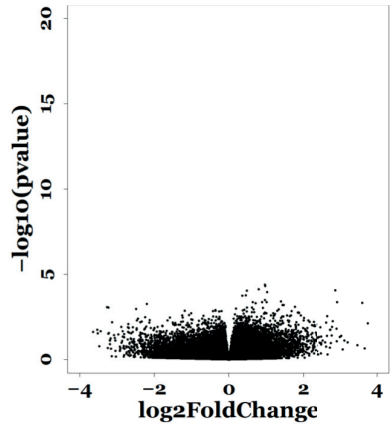
Following the intense weight-loss period (PRE-MID), a significant modulation of the innate immune system was indicated by altered expression levels in regulating key transcription factors and cytokine profile (Figures 18, 19, and 20). In general, suppression of innate immunity was implied by the inhibition (*Q* value < 0.05) of the innate immunity pathway in the leukocyte transcriptomic analysis (Figure 19). Concomitant with the suppression of innate immunity expression profile, promoted proliferation and differentiation of granulocyte/macrophage lineage was also suggested by altered expression level of key transcription factor (e.g., *SPI1*, FDR < 0.05) and cytokine profile (e.g., MCP-1 ↑, MDC ↑, GRO ↑, FDR > 0.05) (Figures 18 and 20). Findings of promoted granulocyte/macrophage lineage proliferation was corroborated by the aforementioned augmentation in neutrophil numbers together with the upregulation (FDR < 0.05) of several genes participating in neutrophil chemotaxis and mobilization (e.g., *CXCR1*, *CXCR2*, *STAT3*, *CSF3R*) (Figure 17 and 18).

Furthermore, evidence of a predominant regulatory/wound-healing macrophage (M2/M3) profile as opposed to classically activated macrophages (M1) was also indicated through a shift towards an anti-inflammatory cytokine profile (e.g., TNF-α ↓, IP-10 ↓, FDR < 0.05; IFN-γ ↓, IL-6 ↓, IL-4 ↑, IL-10 ↑, FDR > 0.05) and upregulation of key regulatory genes (e.g., *CREB1*, *CEBPB*, *STAT3*) (Figures 18 and 20).

a



b



c

Downregulated pathways following the weight loss period in diet group (Time*Group, PRE-MID)			
Pathway description	**Source**	***P* value**	***Q* value**
Metabolism	Reactome	3.73E-09	3.61E-07
Immune System	Reactome	5.96E-08	1.93E-06
Signal Transduction	Reactome	5.96E-08	1.93E-06
Metabolism of proteins	Reactome	9.54E-07	2.31E-05
Post-translational protein modification	Reactome	3.81E-06	7.40E-05
Innate Immune System	Reactome	3.05E-05	4.23E-04
Adaptive Immune System	Reactome	3.05E-05	4.23E-04
Transmembrane transport of small molecules	Reactome	2.44E-04	2.96E-03
Gene Expression	Reactome	4.88E-04	5.26E-03
Deubiquitination	Reactome	1.95E-03	1.72E-02
Hemostasis	Reactome	3.91E-03	2.71E-02
Class I MHC mediated antigen processing & presentation	Reactome	3.91E-03	2.71E-02
Fc epsilon receptor (FCERI) signaling	Reactome	3.91E-03	2.71E-02
Metabolism of lipids and lipoproteins	Reactome	7.81E-03	4.21E-02
Antigen processing: Ubiquitination & Proteasome degradation	Reactome	7.81E-03	4.21E-02

Figure 19. Volcano plots (panel a and b) and most significant pathways (panel c) of gene-level differential expression analysis results. Volcano plots in panels a (PRE-MID) and b (PRE-POST) represent Wald test contrast results from time-point interval comparisons between diet and control groups. Differentially expressed genes (DEGs) with statistically significant *P* values (false discovery rate, FDR < 0.05) (y-axis). Blue colour indicates downregulation, while red indicates upregulation of expression pattern. Magnitude of expression change is depicted on the x-axis with log2FoldChange. Panel c shows most significant pathways associated with the 231 downregulated DEGs from Time*Group interaction after the weight-loss period (PRE-MID).

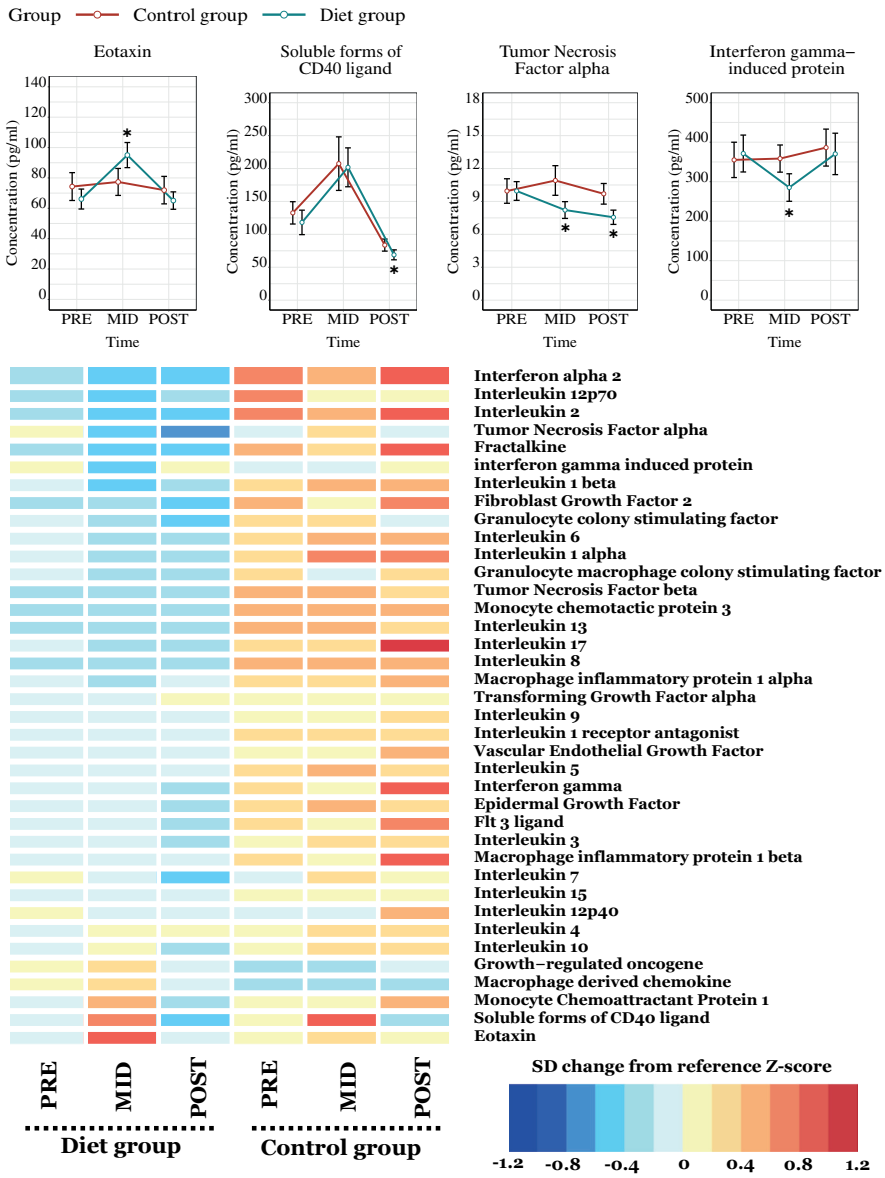


Figure 20. Cytokine profile alteration in the physique study participants during the whole study period. Panel-a line-plots show cytokines that were altered significantly (* = false discovery rate (FDR) <0.05) in a time-dependent manner during the weight-loss period (PRE–MID) in the diet group. Panel-b depicts a heat map of the entire measured cytokine profile also containing those without significant changes (PRE-MID). Cytokine levels are represented as standard deviation (SD) change from the reference Z-score, that was calculated from the pooled baseline Z-score (PRE) of both the diet and control groups. Each individual group/time-point level was compared against the formulated reference value.

Adaptive immune system

Overall, substantial weight loss resulted in the suppression of adaptive immunity by modulating and inhibiting signatures associated with lymphocyte proliferation in both T- and B-cell subsets, as was suggested by leukocyte transcriptomics, cytokine profile, and IgG antibody measures (Figures 18 and 20).

First, similar to attenuated signatures of the innate immune system, overall suppression of adaptive immunity was suggested by downregulation (*Q* value <0.05) of the adaptive immunity pathway activity in the leukocyte transcriptomic analysis (Figure 19). Particularly, suppressed proliferation of two major T cell lines, CD4+ and CD8+ cells, was indicated by differential expression of a multiplicity of key regulating genes (e.g., *GATA3*, *ZBTB7B*, *RUNX3*, *MYB*, *TOX*, *EOMES*, *GZMB*, *FASLG*) (Figure 18). Suppression of CD8+ T cell lineage fate was implied further by significant (*Q* value < 0.05) downregulation of the pathway involved in the Class I MHC mediated antigen processing and presentation (Figures 19).

Second, closer examination of CD4+ T_H subsets implied a shift towards enhanced T_H2 and T_{FH} response, whereas attenuated activation of T_H1 and T_H17 cell lines was suggested by expression levels of associated regulating genes (e.g., *TBX21*, *GATA3, EOMES*, *RUNX3*, *STAT4*, *BCL6*) and cytokines (Figure 18). The suggested predominant T_H2 response was supported further by increased levels of eotaxin ($\beta = 28.95 \pm 5.69$, FDR = 1.36×10^{-5}), a cytokine related to eosinophils that have repeatedly been associated with the effector arm of T_H2 immune responses (Figure 20) [30].

Third, concomitant with the suggested alteration in T cell mediated immunity, altered B cell proliferation and attenuated antibody mediated immunity was also implied as suppressed formation of mature antibody secreting plasma cells was indicated by differential expression of transcription factors (e.g., *BCL6*, *BLIMP*, *XBP1*, *CD27*, *CD38*) and diminished levels of IgG antibodies ($\beta = -0.04 \pm 0.01$, FDR = 4.85×10^{-3}) after the intense weight-loss period (Figure 18). Altered B cell proliferation

was supported further by a modulated BCR signalling pathway and associated inhibitory genes (e.g., *CD22*, *FCGR2B*, *FCRL2*, *FCRL5*) (Figure 18). Furthermore, the marginal zone and germinal zone B cell differentiation towards memory B cells rather than plasma cells was also implied by the differential expression of key regulating genes (e.g., *NOTCH-1/2*, *TCF3*, *BCL6*, *CD40*, *FCRLA*, and *BAFFR*) (Figure 18).

Fourth, suppressed IgG antibody mediated immunity was detected concomitant with overall modulation of IgG glycosylation status – changes associated with pro-inflammatory activity and reduced affinity of IgGs with specific BCRs. Particularly, glycan peaks which contain digalactosylated and sialylated N-glycans without bisecting N-acetylglucosamine (GlcNAc) as well as derived traits were significantly decreased (FDR < 0.05) after the weight-loss period. In contrast, agalactosylated and monogalactosylated glycans and glycans with bisecting GlcNAc significantly increased (FDR < 0.05) in the diet group after the weight-loss period.

Lastly, the above alterations in humoral IgG mediated immunity were observed concomitant with the significant (*Q* value < 0.05) downregulation of the FceRI signalling pathway — the basophil, eosinophil, and mast cell dependent IgE antigen-specific signalling pathway (Figure 19). Altogether, these findings of attenuated IgE and IgG antigen-specific signalling pathways support each other and the perception of suppressed antibody mediated immunity. No long-term effects were detected for innate or adaptive immune-system signatures as observed changes in leukocyte transcriptome, and cytokine profile, together with IgG levels and glycosylation status reverted mostly back to baseline during the weight-regain period (Figures 18, 19, and 20).

5.1.4 Systemic inflammation

Suppressed immune-system signatures following the intense weight-loss period was accompanied by reduced levels of systemic inflammation signatures as was indicated by a uniform decrease in acute inflammation

markers, hs-CRP (β = -0.24 ± 0.07, FDR = 2.44 x 10^{-3}) and α_1-acid glycoprotein (β = -0.16 ± 0.02, FDR = 3.08 x 10^{-13}) (Figure 21). Attenuated levels of immunity mediated inflammation were further implied by nominal augmentation in the levels of anti-inflammatory cytokines (*e.g.,* IL-4, IL-6, IL-10, FDR > 0.05) and attenuated levels of pro-inflammatory cytokines (*e.g.,* TNF-α, FDR < 0.05; Il-1, IFN-γ, IL-12, IL-18, FDR > 0.05.) (Figure 20). Furthermore, augmented levels of anti-inflammatory lipoprotein, HDL-cholesterol (HDL-C) (β = 0.19 ± 0.04, FDR = 4.42 x 10^{-5}) and associated subsets, were detected as a consequence of the substantial weight loss (Figure 21). In contrast with these observations of attenuated systemic inflammation, increased pro-inflammatory activity of IgG was conversely suggested by the IgG glycosylation analyses as described earlier.

5.1.5 Lipoprotein and lipid metabolism

Overall, anti-atherogenic modulation of lipoprotein profile was suggested as a consequence of the substantial weight loss. As mentioned earlier, augmented levels of HDL-cholesterol (HDL-C) (β = 0.19 ± 0.04, FDR = 4.42 x 10^{-5}) and modulation of associated HDL-subpopulations (n = 47) (FDR < 2.0x10^{-16}), that have been previously associated with enhanced atheroprotective functionality of HDL, were observed following weight loss (Figure 21). Specifically, beneficial changes in size, number, and composition of HDL were suggested by i) robust increase of large HDL-metabolites, ii) decrease of small HDL-metabolites, iii) increase in phospholipid content of large HDL-metabolites, iv) increase in cholesterol and cholesterol ester content of large HDL-metabolites, and v) increase in a major structural protein of HDL, apoAI (β = 0.11 ± 0.03, FDR = 4.68 x 10^{-4}) (Figure 21).

Lipid metabolism was further altered in a presumably favourable manner as a cardiometabolically beneficial modulation of serum TG distribution was mostly observed (Figure 21). Following weight loss, substantially lower levels of serum TGs in VLDL (β = 0.07 ± 0.03, FDR =

0.02) together with increased TG content in HDL (β = 0.03 ± 0.007, FDR = 2.08 x 10^{-4}) and LDL (β = 0.03 ± 0.009, FDR = 1.31 x 10^{-3}) lipoproteins were observed. Modulation in the lipoprotein TG distribution did not result in overall changes (FDR > 0.05) in serum lipoprotein TG levels (within VLDL, IDL, LDL, HDL) following the weight-loss period.

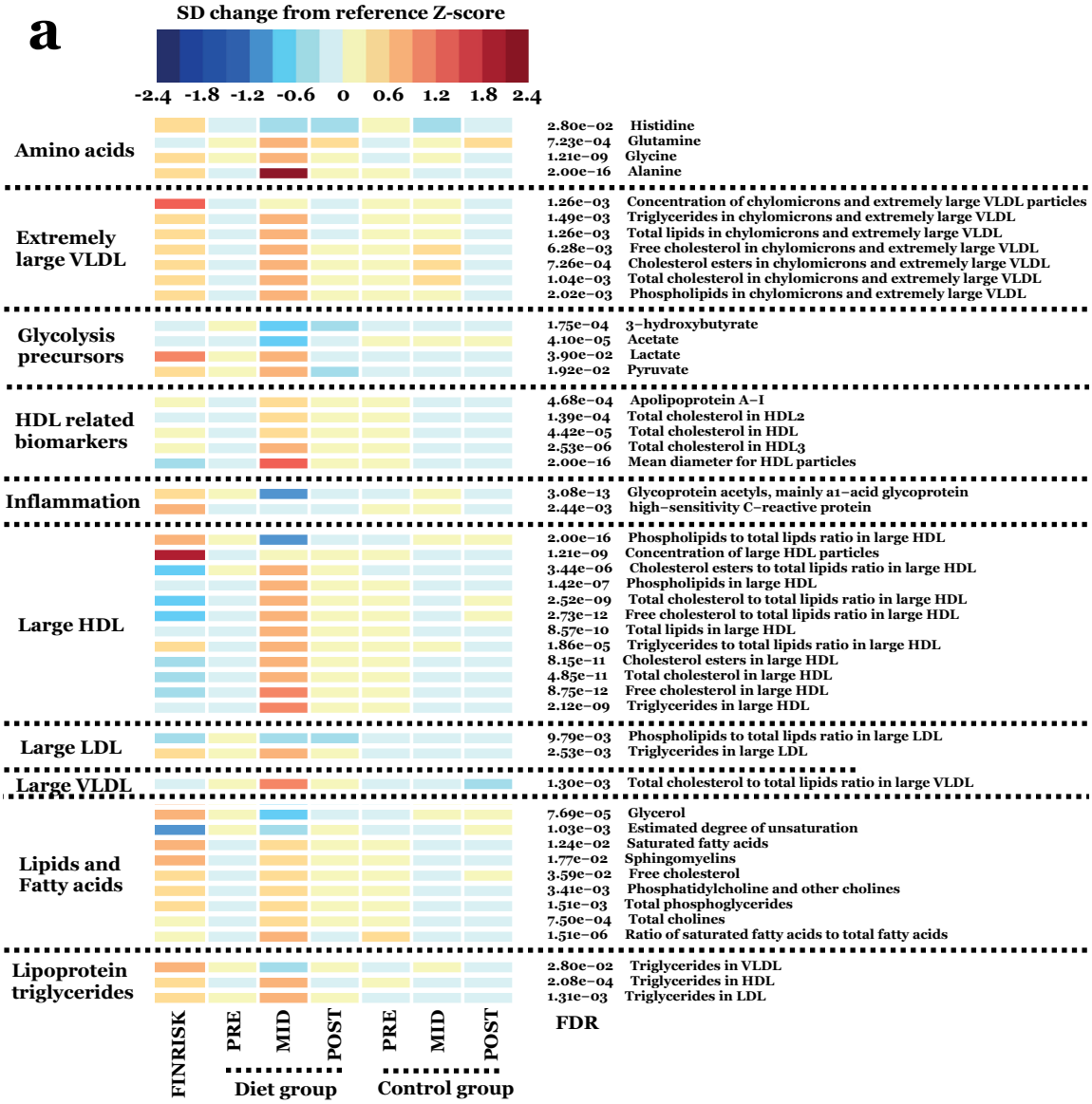
Furthermore, the intense weight-loss period also resulted in potentially cardiometabolically adverse modulation of FFA profiles, as augmented levels of serum SFAs (β = 0.29 ± 0.1, FDR = 0.01) together with overall attenuation in degree of FFA unsaturation (β = -0.05 ± 0.01, FDR = 1.03 x 10^{-3}) was detected. In addition, increased levels of free cholesterol and several lipid/FFA subsets were observed (Figure 21).

5.1.6 Regulation of lipid and lipoprotein metabolism

In accordance with the above metabolomic findings regarding HDL profile and TG levels, DEGs after weight loss were significantly associated (*Q* value < 0.05) with the "Metabolism of lipids and lipoproteins" pathway. Closer inspection of the individual genes in this lipid metabolism pathway revealed augmented expression levels (FDR < 0.0.5) for several genes associated with inhibition of hepatic lipogenesis (e.g., *OSBPL10*), TRL clearance (e.g., *LRP1*, *LPL*), HDL-mediated cholesterol efflux (e.g., *ABCA1*, *ABCG1*, *SCARB1*), regulation of TG metabolism in lipoproteins (e.g., *PPARGC1*), fatty acid transport to cells (e.g., *FATP*), fatty acid synthesis (e.g., *FASN*), and cellular cholesterol metabolism (e.g., *SREB1*, *SREB2*).

Following the intense weight-loss period, significant alteration of (FDR<0.05) enzymatic lipid metabolism regulation was also detected. Specifically, the levels of PON1, anti-inflammatory and -oxidative component of HDL, were increased (β = 2.07 ± 0.89, FDR = 0.048) following the intense weight-loss period, whereas reduced levels of LPL inhibitors, ANGPTL3 (β = -64.55 ± 22.32, FDR = 0.013) and ANGPTL4 (β = -35.92 ± 10.22, FDR = 0.003) were detected, thus potentially contributing to the observed attenuation in TG levels (VLDL-TG).

In addition, a modest increase in PLTP activity (β = 362.29 ± 207.66, FDR = 0.14) was suggested, which is an enzyme associated with increased levels of phospholipid in large HDL particles, consistent with the metabolomics findings of this thesis. Enzyme activities and circulating levels of all measured lipid regulating enzymes/proteins returned close to baseline levels by the end of the weight-regain period.



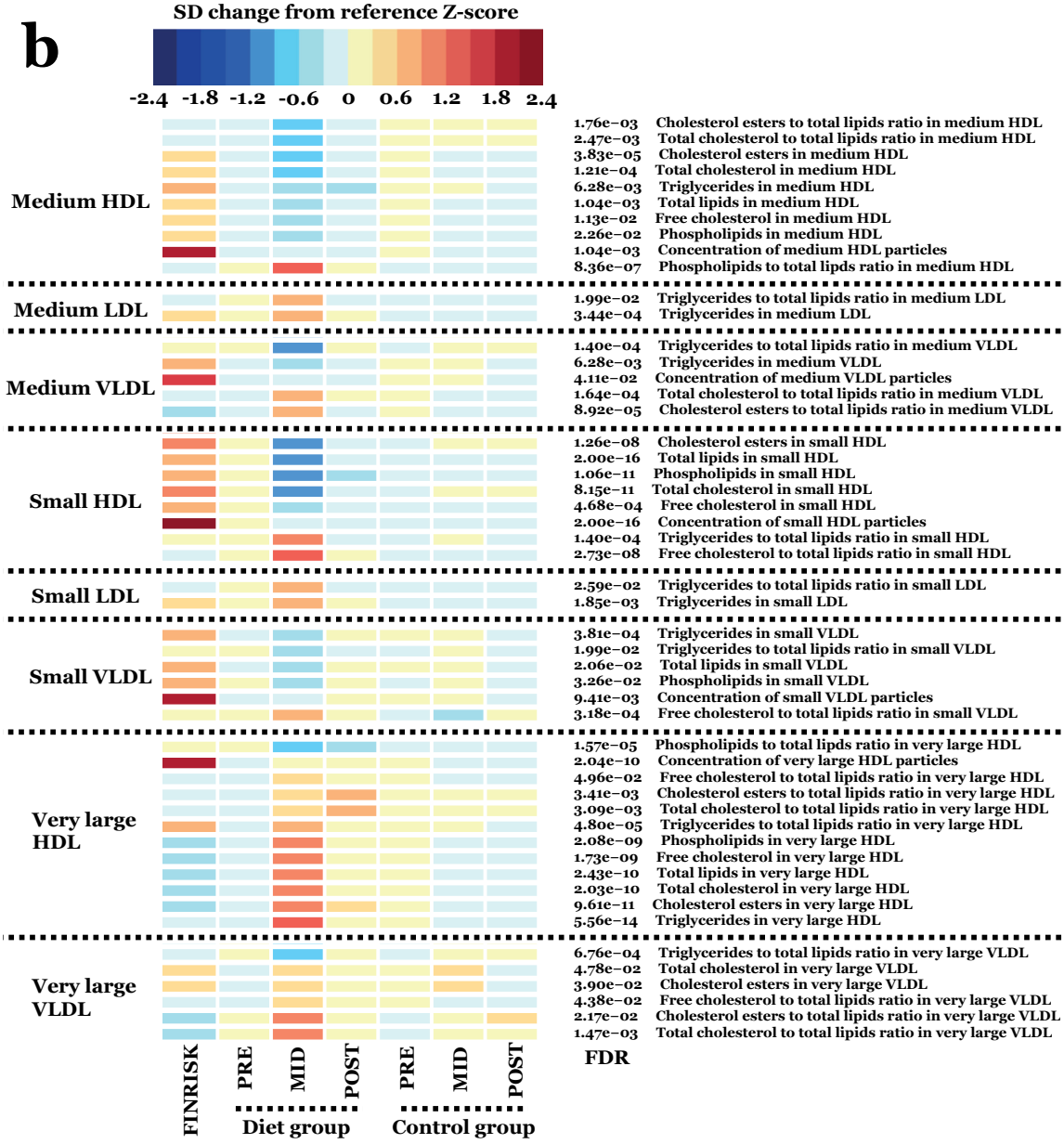


Figure 21. Heatmap of metabolite profile alteration throughout the study period. Metabolite values and colour key are represented as standard deviation (SD) change from the reference Z-score, that was calculated from the pooled baseline Z-score (PRE) of both the diet and control groups. Each individual group/time-point level was compared against the formulated reference value. FINRISK (subsample of DILGOM 2007) represents a subsample of age- and BMI-(propensity)-matched individuals from the general population (n = 58). Multiple testing adjusted *P* values (false discovery rate, FDR) of within diet group analysis after weight loss (PRE-MID) is indicated in front of each metabolite name. Model was defined as follows: metabolite ~ time + age.

5.1.7 Amino acids metabolism

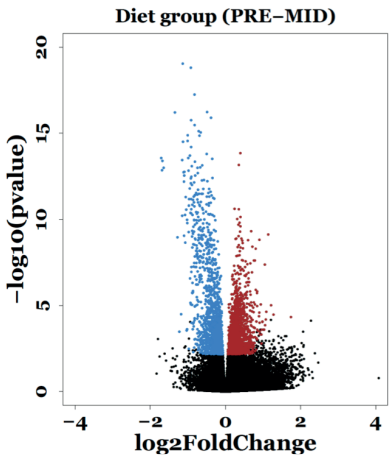
Primarily cardiometabolically beneficial modulation of amino acids profile was also suggested following the intense weight-loss period as increased levels of (FDR < 0.05) alanine, glutamine, and glycine were detected, while serum histidine levels decreased in diet group (Figure 21). No long-term effects were observed in serum amino acids profile as the voluntary weight regain reversed the levels of amino acid close to the baseline levels by the end of the study period (Figure 21).

5.1.8 Systems biology of weight regain and weight cycling

In the study sample of the Female Physique Athlete Study, by the end of the weight-regain period (MID-POST) in the diet group, levels of body weight and fat mass returned back to baseline levels (Table 1). Specifically, 98% of body weight, 87% of total body fat mass, and 90% visceral fat mass were gained back by the end of the study (POST). No wide-array long-term effects were observed in the levels of quantified and analysed system biological factors as the majority of the detected alterations were reverted back to baseline levels during the weight-regain period when exercise volume decreased, energy intake increased, and baseline levels of body fat were obtained.

However, weight regain alone induced a significant upregulation (*Q* value < 0.05) in several pathways related to adverse cardiovascular processes and blood-related signals (e.g., haemostasis, platelet activation, signalling and aggregation, formation of fibrin clot, smooth muscle contraction, dilated cardiomyopathy, and hypertrophic cardiomyopathy) that were detected in the transcriptomic analyses (Figure 22). Interestingly, the preceding weight-loss period had no significant effect on these specific genes and pathways.

a



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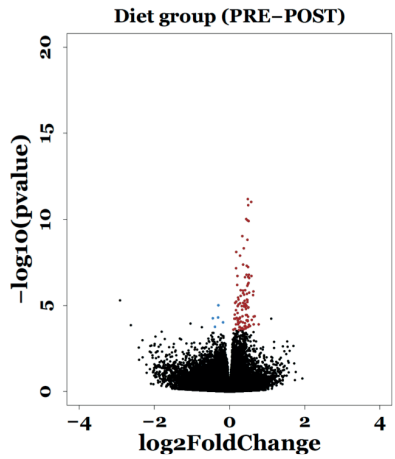


c

Upregulated pathways followng the weight cycling period in the diet grup (Diet group, PRE-POST)			
Pathway description	**Source**	***P* value**	***Q* value**
Platelet degranulation	Reactome	2.23E-12	2.16E-10
Response to elevated platelet cytosolic Ca2+	Reactome	3.37E-12	2.16E-10
Platelet activation, signaling and aggregation	Reactome	5.67E-10	2.42E-08
Hemostasis	Reactome	3.24E-09	1.04E-07
Smooth Muscle Contraction	Reactome	2.21E-07	5.65E-06
Common Pathway of Fibrin Clot Formation	Reactome	1.44E-06	3.06E-05
Molecules associated with elastic fibres	Reactome	2.12E-04	2.71E-03
Hypertrophic cardiomyopathy (HCM) - Homo sapiens (human)	KEGG	3.03E-04	3.52E-03
Elastic fibre formation	Reactome	3.37E-04	3.60E-03
Gap junction - Homo sapiens (human)	KEGG	3.79E-04	3.62E-03
Dilated cardiomyopathy - Homo sapiens (human)	KEGG	3.95E-04	3.62E-03
Cytokine-cytokine receptor interaction - Homo sapiens (human)	KEGG	5.42E-04	4.59E-03
Non-integrin membrane-ECM interactions	Reactome	5.80E-04	4.59E-03
Chaperonin-mediated protein folding	Reactome	6.15E-04	4.59E-03
Signal Transduction	Reactome	6.46E-04	4.59E-03

Figure 22. Volcano plots (panel a and b) and most significant pathways (panel c) of gene-level differential expression analysis results. Volcano plots in panel a (PRE-MID) and b (PRE-POST) represent Wald-test contrast results from time-point interval comparison within diet group. Differentially expressed genes (DEGs) with statistically significant *P* values (false discovery rate, FDR < 0.05) (y-axis). Blue colour indicates downregulation, while red indicates upregulation of expression pattern. Magnitude of expression change is depicted on the x-axis with log2FoldChange. Panel c depicts most significant upregulated pathways associated with 82 DEGs (panel b) affected by the whole weight-cycling period in the diet group (PRE-POST), although these genes were affected only by the weight-regain period, not by weight loss. No significant down-regulated pathways were observed from the 5 downregulated DEGs after the weight-cycling period (PRE-POST).

5.1.9 Comparison of cardiometabolic signatures of physique athletes and matched general population individuals

To determine the differences between individuals with years of training experience and a healthy lifestyle (i.e., physique athletes), and females from the general population of a similar age and BMI, metabolomic profiles from all physique athletes (n = 42) at baseline were compared with BMI- and age-matched individuals (n = 58) from the National DILGOM 2007 Study (Table 1). The comparison revealed substantial differences in the levels of 129 metabolites (FDR < 0.05) between the two groups. Even preceding the weight-loss period, lipid profiles, inflammation markers, and other health-related biomarkers (e.g., α_1-acid glycoprotein, CRP, serum total TGs and cholesterol, degree of unsaturation) were at a more favourable level in the physique athletes compared to the matched general-population individuals (Figures 21 and 23).

Despite being matched based on age and BMI, the two groups had distinct differences in body composition (FDR < 0.05) as the physique athletes had ~22% less fat mass, ~3% smaller waist to hip -ratio, and ~7% higher levels of lean mass when compared to the matched DILGOM 2007 Study individuals (Table 1). The physique athletes also had ~43% higher physical activity than the matched general-population individuals (Table 1). Consistent with the findings in the physique athletes, body composition differences more efficiently predicted cardiometabolic profiles compared to reported levels of exercise levels and dietary intake. However, accounting for differences in body composition dissipated only some of the significant signals in the between-group comparisons.

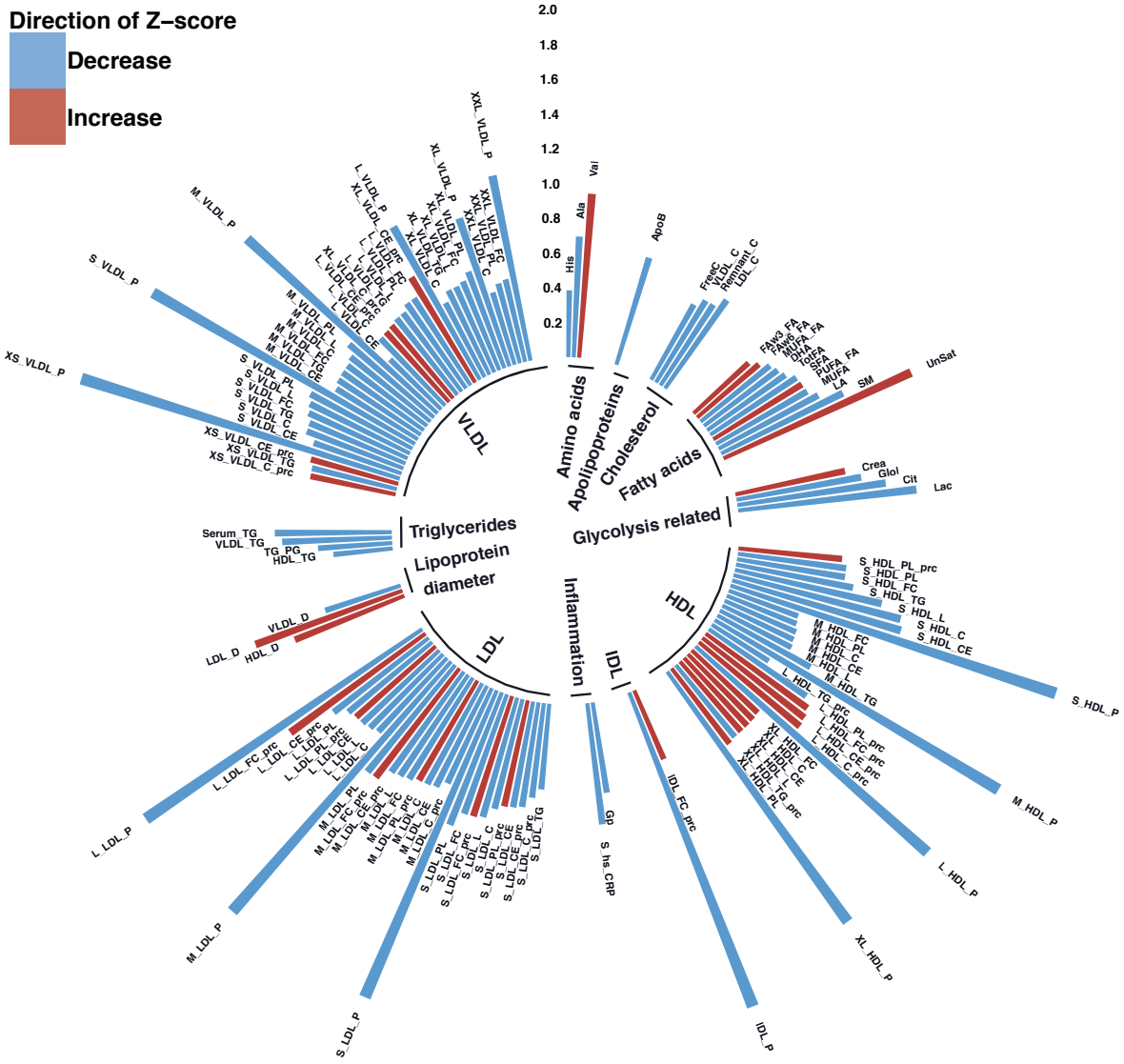
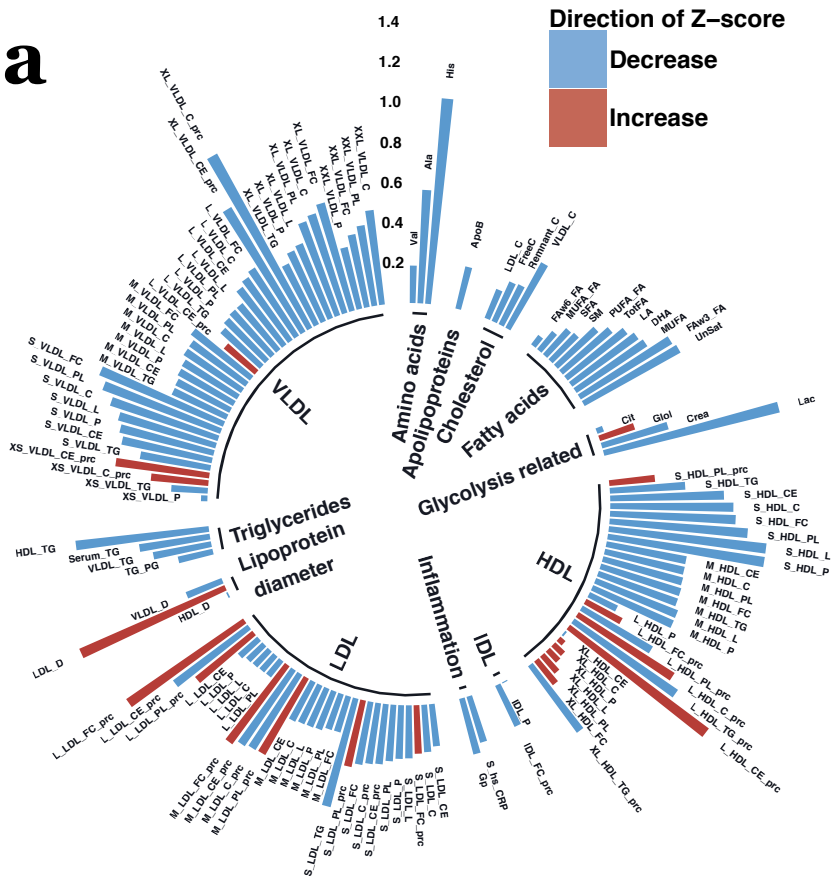


Figure 23. Polar bar plots of metabolome differences between physique athletes and a subsample of DILGOM 2007 Study participants. The levels of 129 health-related biomarkers that differed significantly between the physique athletes and DILGOM 2007 Study subset were plotted to illustrate how an age- and BMI-matched subset of general population metabolite profile is altered compared to the physique athletes. Polar plots are derived from metabolite raw-values excluding outliers that were 4 standard deviations (SD) from the mean. Metabolite values are depicted as SD change from the reference Z-score. As a reference, Z-score metabolite values of DILGOM 2007 Study individuals were utilized to which physique athletes' values were compared. Participants from both physique athlete groups (diet and control group, n = 42) were pooled together at baseline (PRE) to increase sample size when compared to age- and BMI-matched general population DILGOM 2007 Study individuals (n = 58).

5.1.10 Cardiometabolic signatures of weight loss and gain in the general-population normal-weight individuals

In the end, long-term weight loss and gain was investigated in the subset of DILGOM 2007 Study individuals who had altered adiposity (n = 20) after a 7-year follow-up to determine associated changes in cardiometabolic profile (Figure 24). Consistent with the findings from physique athletes, fat mass loss of ~10% (n = 7) resulted in similar cardiometabolically-beneficial alteration in inflammation markers, serum cholesterol, lipid distribution, and lipoprotein composition in the matched general-population individuals (Figures 21 and 24). Furthermore, fat mass gain of ~20% (n = 13) induced adverse changes in cholesterol levels, lipoprotein distribution, and composition similar to the changes observed during the weight-regain period in the physique athletes (Figures 21 and 24).



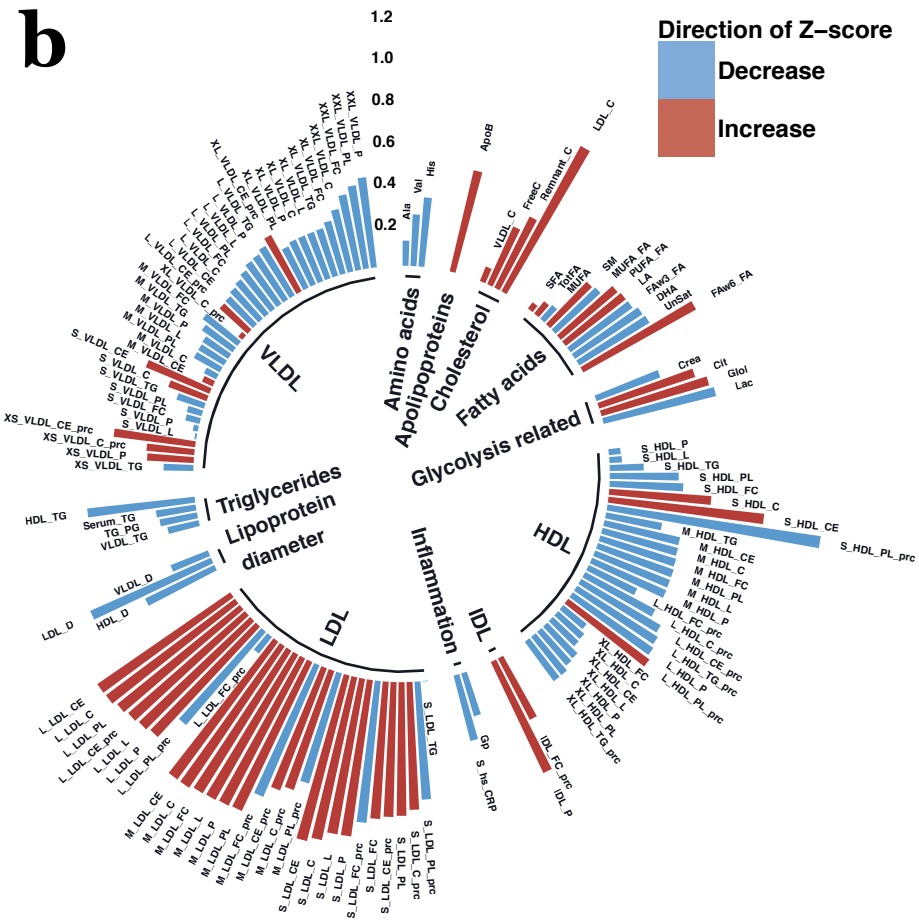


Figure 24. Polar bar plots of metabolome differences among DILGOM 2007 Study participants who lost or gained weight during the 7-year follow-up (n=20). A subset of normal-weight DILGOM 2007 Study individuals who lost weight (n = 7, panel a) and gained weight (n = 13, panel b) during a 7-year follow-up was explored to determine if cardiometabolic signatures were affected in a similar manner as within the physique athletes following weight loss and weight gain. For plotting, the same 129 metabolites were utilized that were significantly altered between the physique athletes and DILGOM 2007 Study subset. Polar plots are derived from metabolite raw-values excluding outliers that were 4 standard deviation (SD) from the mean. Metabolite values are depicted as SD change from the reference Z-score. DILGOM 2007 Study individual baseline information (2007) was set as the reference Z-score to which the follow-up metabolome profile of 2014 was compared to. Although, similar patterns of weight loss and weight regain on cardiometabolic signatures were suggested – only nominally significant findings were detected due to small sample size and subsequent limited statistical power.

5.2 The Male Resistance Training Study: Cardiometabolic signatures of resistance training and lean mass

5.2.1 Short-term resistance training effect on body composition

In the Male Resistance Training Study, a 16-week RT regimen (PRE- to POST-16wk) resulted in overall favourable alterations in body composition. As shown in Table 2, the RT intervention lead to ($P < 0.05$) increased levels of total lean mass (~2.8%), decreased levels of visceral (~9.6%), and total fat mass (~7.5%) in the RT group only.

Closer examination of the study population revealed high- [the greatest quartile, change 5.9 (1.9) %, n = 15] and low-responders [the lowest quartile, change 0.3 (0.7) %, n = 14] that were detected based on the relative lean mass (normalized to BSA, $kg \cdot m^2$) gained during the RT intervention (PRE- to POST-16wk). The lean mass high-responders were also more susceptible to the effects of RT-induced fat mass compartment alteration. Hence, body composition of high-responders was further altered in a beneficial manner as increased levels of gained lean mass were detected together with significant reductions in visceral fat mass (~20.6%) and relative overall adiposity (~17.7%). Baseline phenotype differences between responder groups could be observed also on lean mass, fat mass, and dietary intake (Table 2).

5.2.2 Resistance training effects on metabolomic profile

Overall, longitudinal metabolomic analysis revealed that the RT group had significant (FDR < 0.05) differences in the levels of 21 metabolites between baseline and follow-up (PRE- to POST-16wk). The favourable changes in body composition presumably explained to some extent the suggested anti-atherogenic alterations in serum metabolome profile (FDR < 0.05), where reductions in non-HDL cholesterol (e.g. free cholesterol, remnant cholesterol, IDL cholesterols, LDL cholesterols) and related apoB, and increments in CLA levels were observed as depicted in Figure 25. In

addition, augmented levels of aromatic amino acids, phenylalanine and tyrosine, and glutamine were detected (FDR < 0.05) in response to the RT period.

5.2.3 Responder status effect on metabolomic profile

The status of lean mass gain high- and low-responders was distinctively reflected on the overall biomarker profile as more wide-array effect on the serum lipoprotein levels were observed in high-responders when compared to low-responders (Figure 26). Particularly, these differences in response to RT intervention were most evident in some of the HDL subclasses that increased distinctively in the high-responder group as opposed to low-responders (PRE- to POST-16wk) (Figure 26). Interestingly, however, low-responders seemed to have higher levels of HDL metabolites (FDR > 0.05) at baseline, which might have contributed somewhat to the observed difference across time points between responder groups.

In the end, the levels of five different large-HDL metabolites were modulated significantly (FDR < 0.05) across time points between high- and low-responders, where increments in lean mass explained most strongly these differences in HDL profile (Figure 27). Moreover, discrepancies in overall adiposity did not further explain detected differences in HDL profile, thus further highlighting the signal between lean mass gains and HDL profile.

Table 2. Descriptives of participants who responded differentially to resistance training intervention (defined by gain in lean mass) (n = 59).

	Low responder			Median responder			High responder		
	Baseline	**POST-4wk**	**POST-16wk2**	**Baseline**	**POST-4wk**	**POST-16wk**	**Baseline**	**POST-4wk**	**POST-16wk**
N	14	14	14	30	30	30	15	15	15
Age	35.8 (5.4)	35.8 (5.4)	35.8 (5.4)	32.4 (6.7)	32.4 (6.7)	32.4 (6.7)	32.9 (8.6)	32.9 (8.6)	32.9 (8.6)
BMI (kg/m^2)	24.1 (2.3)	24.4 (2.4)*	24.1 (2.3)	23.5 (3.3)	23.6 (3.2)	23.5 (3.3)	24.6 (1.8)	24.4 (1.7)	24.6 (1.8)
Fatmass index (kgm^2)	9.5 (3.7)	9.7 (3.8)	9.6 (3.7)	9.1 (4)	8.8 (3.9)*	8.6 (3.8)*	10.8 (2.6)	10 (2.8)*	8.9 (2.4)*
Leanmass index (kgm^2)	29.6 (2.2)	29.6 (2.4)	29.7 (2.2)	29.5 (2.1)	29.8 (2.2)*	30.2 (2.1)*	28.6 (2)	29.4 (2.2)*	30.3 (2)*
Android fatmass (kg)	2.2 (1.1)	2.2 (1.1)	2.1 (1.1)	2.1 (1.2)	2.0 (1.1)*	1.9 (1.1)*	2.3 (0.8)	2.0 (0.8)*	1.8 (0.7)*
Legpress 1RM ¶	3.7 (0.4)	4 (0.4)	4.3 (0.6)	3.5 (0.6)	3.7 (0.6)*	4 (0.6)*	3.4 (0.5)	3.5 (0.5)*	3.8 (0.4)*
Total energy (kCal/kg/d) ¶	†	35.3 (8)	†	†	32.1 (8.4)	†	†	26.2 (7.2)	†
Protein (g/kg/d)	†	1.8 (0.5)	†	†	1.6 (0.4)	†	†	1.4 (0.3)	†
Carbohydrate (g/kg/d) ¶	†	3.5 (1)	†	†	3.3 (1.2)	†	†	2.6 (0.9)	†
Fat (g/kg/d)	†	1.3 (0.3)	†	†	1.2 (0.3)	†	†	0.9 (0.3)	†

* = Statistically significant ($P < 0.05$) difference compared to baseline within different responder groups. ¶ = Statistically significant difference ($P < 0.05$) between low-responders and high responders at baseline. No other significant differences were observed between responder groups at baseline. † = Dietary information with food diaries (3+1 days) was gathered only after the post 4-wk time point. Participants were instructed to maintain their usual dietary behaviour and intake throughout the study period. Dietary information was available for n = 38 participants from the intervention group (n = 59). Leg press 1 repetition maximum (1RM) was normalized to total lean body mass.

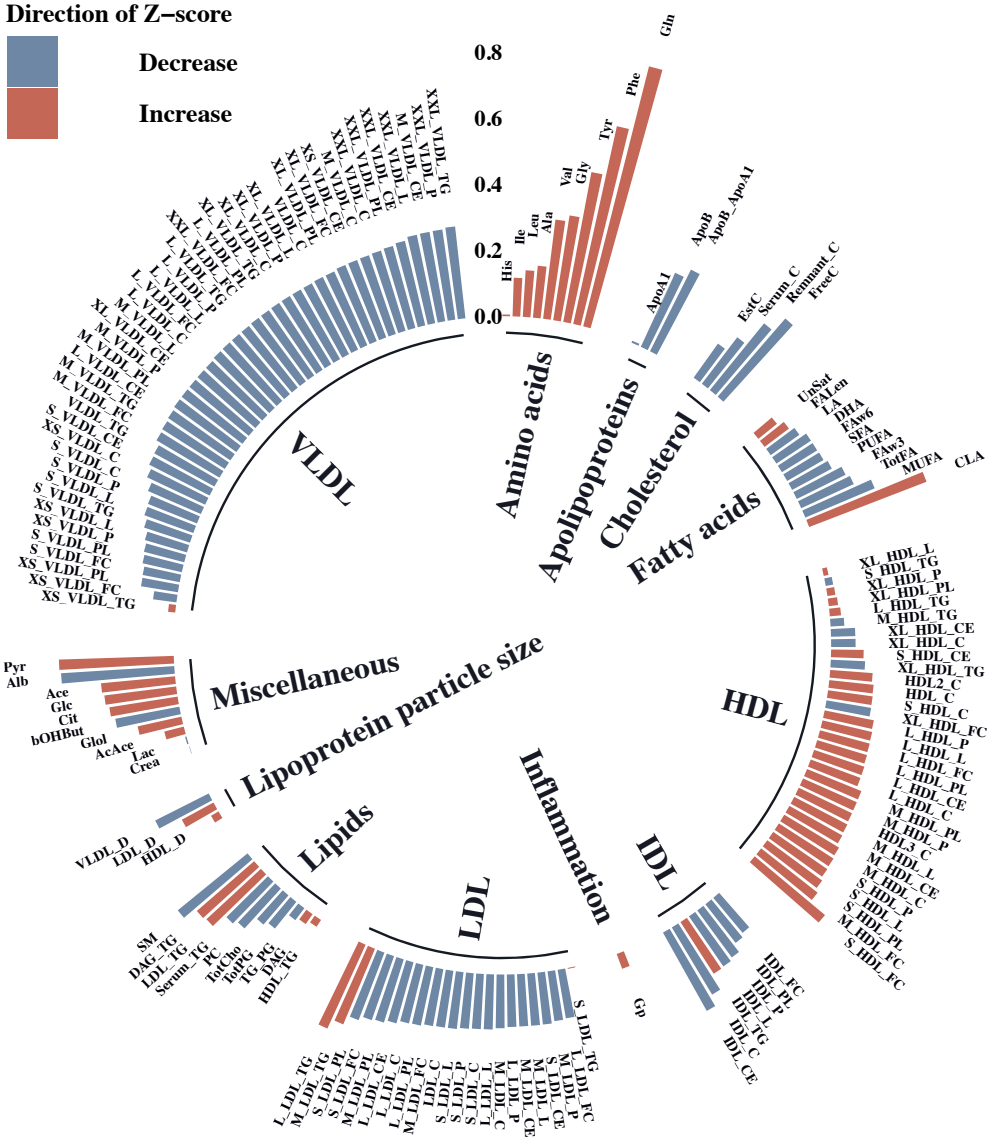
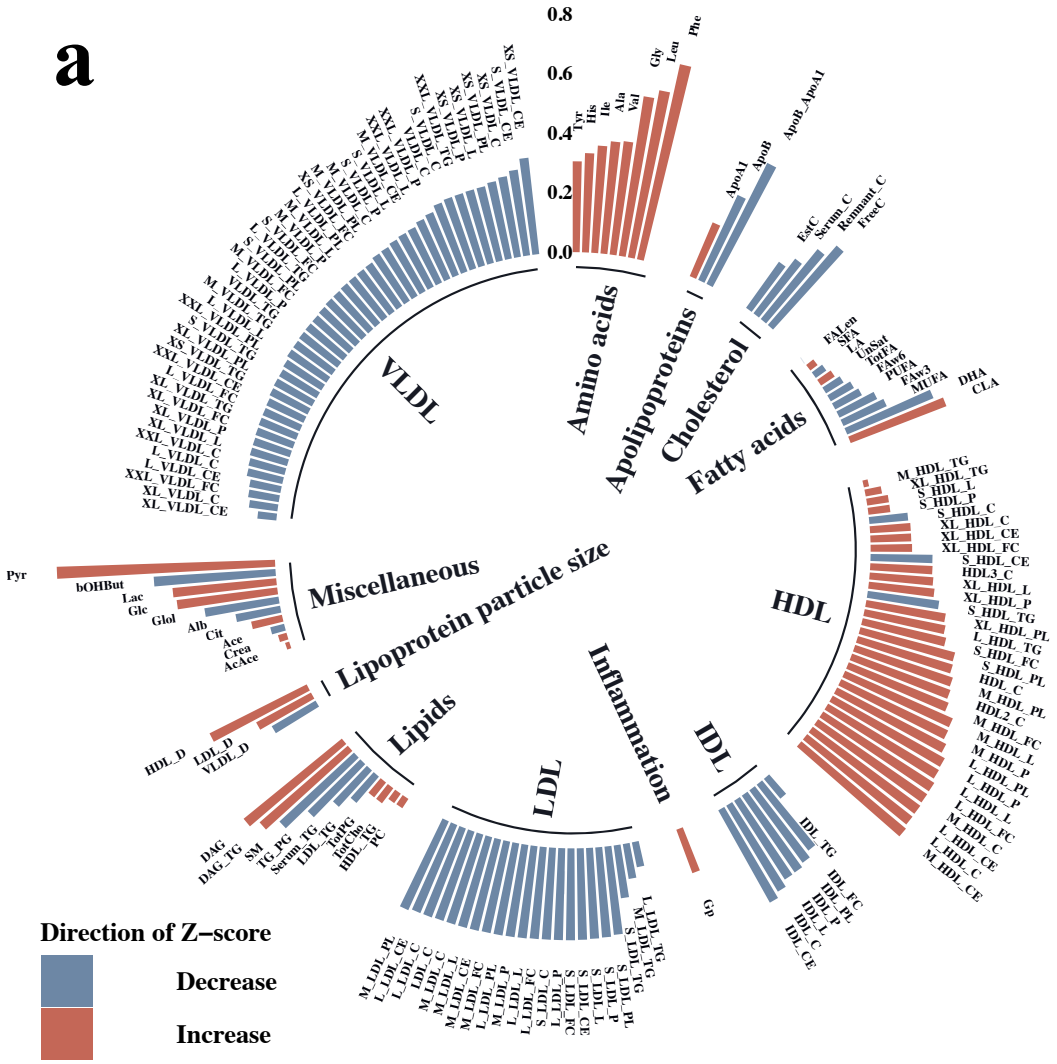


Figure 25. Overall changes in the metabolome profile after the 16-week resistance training intervention. For plotting, 155 health related biomarkers with absolute levels were selected to illustrate the effect of the 16-week resistance training intervention (n=59) on overall metabolome profile. Depicted polar plot is derived from metabolite raw-values where outliers based on 4 standard deviation (SD) from mean have been excluded. Plotted metabolite values are represented as SD change from set reference Z-score, where baseline values were set as a reference level. Height of the bars depicts the level of Z-score. Scale is plotted on the apex of the figure vertically.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0.8
0.6
0.4
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Amino acids
Tyr
His
Ile
Ala
Val
Gly
Leu
Phe
Apolipoproteins
ApoA1
ApoB
ApoB_ApoA1
Cholesterol
EstC
Serum_C
Remnant_C
FreeC
Fatty acids
FALen
SFA
LA
UnSat
TotFA
FAw6
PUFA
FAw3
MUFA
DHA
CLA
HDL
M_HDL_TG
XL_HDL_TG
S_HDL_L
S_HDL_P
S_HDL_C
XL_HDL_C
XL_HDL_CE
XL_HDL_FC
S_HDL_CE
HDL3_C
XL_HDL_L
XL_HDL_P
S_HDL_TG
XL_HDL_PL
L_HDL_TG
S_HDL_FC
S_HDL_PL
HDL_C
M_HDL_PL
HDL2_C
M_HDL_FC
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M_HDL_P
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L_HDL_L
M_HDL_FC
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L_HDL_C
M_HDL_C
M_HDL_CE
IDL
IDL_TG
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Inflammation
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LDL
L_LDL_TG
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Lipids
HDL_D
LDL_D
VLDL_D
DAG
DAG_TG
SM
TG_PG
Serum_TG
LDL_TG
TotPG
TotChol
HDL_TG
PC
Lipoprotein particle size
VLDL
XS_VLDL_CE
S_VLDL_CE
XS_VLDL_C
XS_VLDL_PL
XS_VLDL_P
XS_VLDL_L
XS_VLDL_TG
XXL_VLDL_C
S_VLDL_C
XXL_VLDL_TG
XXL_VLDL_P
XXL_VLDL_L
XXL_VLDL_CE
M_VLDL_CE
S_VLDL_L
S_VLDL_P
M_VLDL_FC
M_VLDL_C
S_VLDL_TG
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M_VLDL_L
M_VLDL_P
M_VLDL_TG
L_VLDL_PL
L_VLDL_P
L_VLDL_TG
XXL_VLDL_PL
XS_VLDL_TG
L_VLDL_CE
XXL_VLDL_FC
XL_VLDL_TG
XL_VLDL_FC
XL_VLDL_P
XXL_VLDL_L
L_VLDL_C
L_VLDL_CE
XXL_VLDL_FC
XL_VLDL_C
XL_VLDL_CE
Miscellaneous
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bOHBut
Lac
Glc
Glol
Alb
Cit
Ace
Crea
AcAce
Direction of Z−score
Decrease
Increase

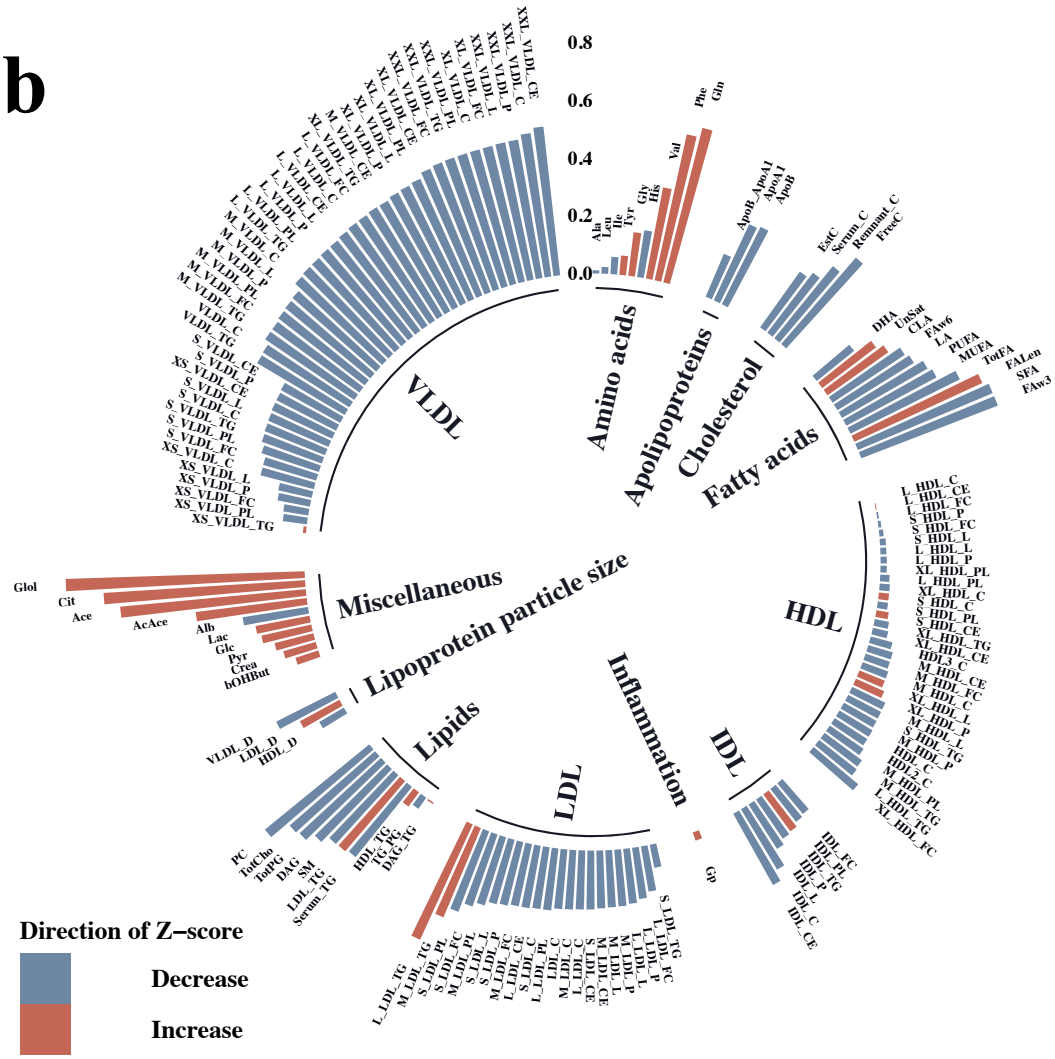

Figure 26. Overall differences in the metabolome profile after the 16-week resistance training intervention between low- and high responders. Similar to Figure 23, 155 health related biomarkers were selected to demonstrate the overall differences on cardiometabolic signatures between low- and high-responders in response to the 16-week resistance training intervention. Panel a depicts the changes in high-responders (n = 15) whereas panel b demonstrates the changes in metabolome profile in low-responders (n = 14) following the resistance training intervention (PRE–POST-16wk). Depicted polar plots are derived from metabolite raw-values where outliers based on 4 standard deviation (SD) from mean have been excluded. Plotted metabolite values are represented as SD change from set reference Z-score. Baseline metabolite values were used as a reference Z-score.

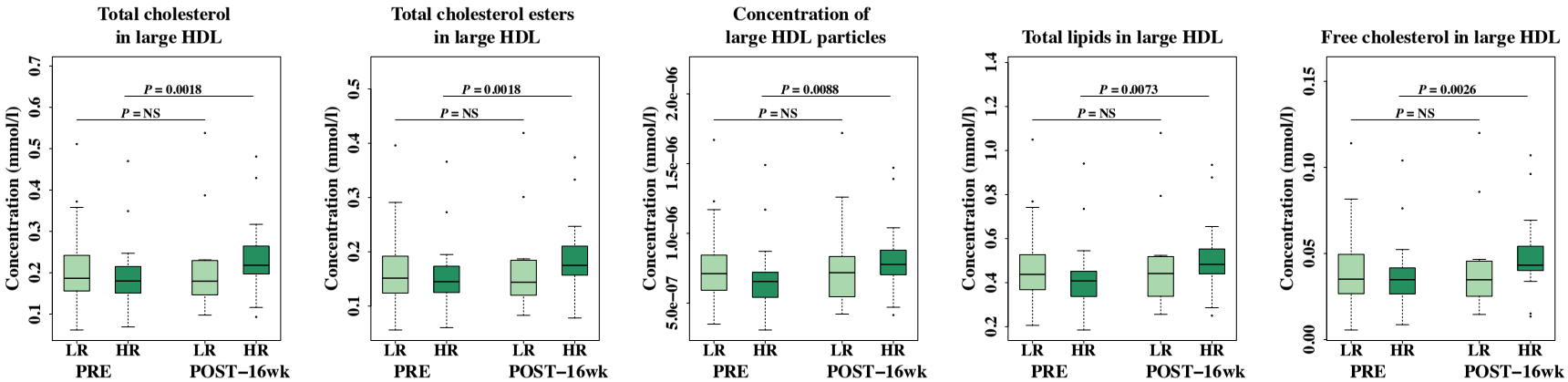

Figure 27. Most significant metabolite changes between high- and low-responders relative to lean mass change following the 16-week resistance training intervention. Figure demonstrates most significant metabolite changes (PRE- to POST-16wk) within the resistance training group when divided into high- (n = 15) and low-responders (n = 14) based on highest and lowest quartile of lean mass index change. LR = Low-responder. HR = High-responder. PRE = Baseline. POST-16wk = After 16 weeks of intervention. NS = Not significant (*P* value < 0.05). Depicted boxplots are derived from 4 standard deviations (SD) quality-controlled data.

6 DISCUSSION

6.1 Systems biology of weight loss, exercise training, and low-energy availability

The results of this thesis demonstrated for the first time that even in healthy, normal-weight individuals, further reduction of body and visceral fat mass results in positive changes in anti-atherogenic HDL profile, lipid levels, and inflammation-related biomarkers. These cardiometabolic effects were accompanied by evidence of immunosuppression and dysregulated haematopoiesis as a consequence of prolonged low energy availability and intense exercise training. No previous studies examining the effects of intense transient weight loss in women have been conducted before. Similar focus has been reported only once in men, in the 1940s in a seminal experiment that investigated the effects of a long-term extreme low-calorie diet by subjecting young normal-weight men to a semi-starvation treatment for 24 weeks [193,194].

It was also demonstrated that even during *ad libitum* diet, RT has the potential to induce favourable alterations in body composition together with serum non-HDL and lipid profile even in healthy normal-weight individuals. The findings of this thesis also characterised and suggested that individuals with the poorest baseline phenotype and cardiometabolic status benefit the most from RT regimens.

Haematopoiesis and bone metabolism

Dietary energy restriction has been elucidated as an important promoter of HSC quiescence, whereas, in contrast, physical exercise training has been shown to be one of the most potent stimuli for the opposite effects of HSC proliferation from bone marrow to the circulation [90,195,196]. It can be speculated that loss of quiescence and excessive proliferation of HSCs through a very high amount of exercise training with energy restriction, observed in dieting physique athletes, may accelerate bone marrow HSC proliferation, leukocyte release to circulation, and thus the exhaustion of

HSC niches [89,197-199]. Subsequently, in concordance with the findings of this thesis, aging phenotypes of HSCs have been characterised by i) an increase in the pool size of HSCs, ii) skewing toward myeloid-biased HSCs versus lymphoid-biased HSCs, iii) enhanced leukocyte mobilization from the bone marrow into the circulation, and iv) suppressed erythroid lineage proliferation [89]. Furthermore, the suggested enhanced HSC proliferation and turnover from bone marrow was also corroborated by the overall loss of bone mass after substantial weight loss that was reported in the earlier published study from these competitors [16]. Based on previous studies and the findings of this thesis, it can be argued that the combined prolonged stress of high-volume training and low energy availability without sufficient recovery leading to increased metabolic stress might have the potential to enforce HSC proliferation resulting in altered circulating levels of leukocytes.

Innate immune system

Lean phenotype, voluntary weight loss, and anti-inflammatory skewed cytokine profiles, together with enhanced CD4 T_H2 responses as suggested in the Female Physique Athlete Study (paper II), have been associated with the alteration of innate immune cells with a regulatory/wound-healing macrophage (M2/M3) dominant profile [27,200-202]. Regulatory/wound-healing macrophages have the ability to dampen immune-system responses and limit inflammation [27], thus reinforcing further the suggestion of alleviated systemic inflammation and immunosuppression after the intense weight-loss period in the normal-weight female athletes. However, although both classically activated macrophages (M1) and associated pro-inflammatory cytokine flux have been linked with adverse health effects such as chronic diseases, obesity, and disease related weight loss, they also mediate positive effects in the eradication of premalignant tumour cells [27,203,204]. Thus, these observations highlight the delicate existing homeostasis that needs to be sustained between immune-system activation

and suppression to prevent adverse health effects. Despite the beneficial direction of changes observed in the systemic inflammation and immune responses of the adaptive and innate immune system in this thesis, it remains to be elucidated whether these characterised alterations mediate additional health benefits or whether they lead to adverse disruption of immune system homeostasis by excessive immunosuppression.

Adaptive immune system

Malnutrition, nutritional deprivation, and low-energy availability are considered to be key causes of promoting immunodeficiency. The most common immune defects are atrophy of lymphoid tissues (e.g., thymus), reduced maturation of T cells, an imbalance in the ratio of CD4/CD8 T cells, and predominant T_H2 helper cell response [125,205-209]. These alterations were consequently suggested by the leukocyte transcriptome and cytokine profile in the Female Physique Athlete Study (paper II) of this thesis. These immunosuppression-associated immune-system defects have been previously reported to be mediated by diminished levels of leptin caused by both low-energy availability and exhaustive exercise [16,210]. Consequently, leptin administration and moderate levels of exercise have successfully prevented these immune system alterations and following immunosuppression [125]. In accordance with these observations from previous studies, a reduced volume of exercise and increased energy intake leading to weight regain, and previously published elevation in leptin levels [16], normalized signatures of the transcriptomic regulation of T cell proliferation and T_H cell response in the Female Physique Athlete Study (paper II).

Altogether, it can be speculated that energy restriction and exercise-induced reduction in leptin levels may mediate suppressed peripheral T cell proliferation and predominant T_H2 helper cell response even in humans, thus promoting the immune-system alteration associated with immunodeficiency. However, additional studies with similar study design

should be pursued where the changes in lymphocyte proliferation and lymphocyte subset number should be assessed with more specific methods (e.g., flow cytometry, proteome) [211].

Previous studies also suggest that low-energy availability negatively impacts B cell maturation, however, the underlying mechanisms have not been characterised in detail in humans. It has been implied that both starvation and HSC aging affect B cell maturation in various ways, including i) to arrest B cell development, ii) to diminish the pool of naive B cells, and iii) to expand the GC cell pool of memory B cells [80]. Subsequently, similar findings were suggested in the Female Physique Athlete Study (paper II), as evidence of suppressed B cell proliferation was detected in addition to skewed-GC expansion, diminished production of mature IgG producing plasma cells, and reduced IgG antibody production. To further corroborate these findings, a similar diminished antibody production has been observed in aging immune systems and in highly trained athletes during prolonged periods of intensive exercise training without sufficient recovery [212].

Suppression of signatures associated with B cell proliferation and secretion function in response to a prolonged period of low energy availability have been proposed to be mediated by the previously discussed alteration in leptin levels [119,209]. In the end, it can be speculated that reduction in leptin levels may at least in part also contribute to these modulations in B cell maturation, and subsequent antibody secretion. Therefore, the findings in this thesis reinforce the perception that the impact of prolonged dietary restriction and a very high volume of exercise training may also extend to the proliferation and function of the B cell population, thus contributing to mechanisms behind immunosuppression following substantial weight loss in normal-weight individuals.

Autoimmunity

In the past, severe dietary restriction and malnutrition in individuals with low body weight (e.g., anorexia nervosa) has been associated with increased risk of number of autoimmune diseases [213]. In these individuals, mucosal

abnormalities/disturbances including thinned mucosa, infiltration of immune cells, and increased intestinal permeability have been characterised [112]. Consequently, increased pro-inflammatory activity of IgG (galactosylation ↓, bisecting GlcNAc ↑), reduced affinity of IgGs (sialylation ↓) with specific BCRs (e.g., FCRLs), altered IgE mediated signalling, higher levels of eotaxin, and predominant T_H2 response were observed in the Female Physique Athlete Study (paper II). These immune system modulations have all been previously associated with dysregulation of the immune system and autoimmune diseases related to the lungs and intestines, such as asthma, inflammatory bowel disease, and gastrointestinal allergic hypersensitivity [101,214]. Alterations in these molecular pathways associated with mucosal immunity could to some extent explain the mechanism/pathophysiology behind higher risk of infection (e.g., upper respiratory tract infections) during periods of very high volume of exercise training with low-energy availability, especially observed among highly trained athletes [210,215-218]. Altogether, multiple aspects of suppressed immune-system signatures, together with findings related to autoimmunity, provoke the question as to whether these immune-system related alterations predispose to greater risk of autoimmune dysregulation and adverse immune-system–associated health outcomes in the long run, i) when repeated bouts on substantial weight loss preceding competition are undergone, and ii) if insufficient time is allowed for recovery between diets [219].

Systemic inflammation

Cardiometabolically-beneficial modulation of anti-inflammatory lipoprotein HDL, together with the reduced levels of inflammatory metabolomic markers (e.g., α1-acid glycoprotein, hs-CRP) and cytokines (e.g., TNF-α, IP10) following weight loss, suggested attenuated levels of systemic inflammation in normal-weight individuals undergoing substantial weight loss in the Female Physique Athlete Study (paper I).

Similar changes have also been observed in overweight individuals, where weight loss has been shown to attenuate and normalize augmented levels of systemic inflammation markers [220].

It is widely accepted that obesity, adipose tissue dysfunction, and weight gain, together with elevated levels of systemic inflammation, have also been associated with elevated levels of the total number of leukocytes (e.g., neutrophils) [96,97]. Although, most often used as an indicator of infection, an increased number of leukocytes has also been characterised as a predictor of systemic low-grade inflammation and coronary heart disease [221]. Subsequently, augmented levels of neutrophils were detected in consequence to the substantial weight loss in the Female Physique Athlete Study (paper II), finding in contradiction with the observed attenuated levels of other inflammation markers and previous weight-loss studies among overweight individuals [220]. Weight loss below normal levels of fat mass alone did not seem to explain this disparity, as disease-related weight loss (e.g., anorexia nervosa) with similar drastic drop in weight and fat mass has been conversely characterised with increased levels of inflammatory markers but reduced levels of leukocytes [203]. These observations together with the findings of this study, highlight and reinforce the perception that starting weight and possibly also the way in which weight loss is achieved (energy restriction vs. exercise mediated, exercise training volume vs. intensity) have a considerable effect on the markers of systemic inflammation.

To conclude, beneficial alteration of blood-derived metabolomic and cytokine markers alleviated doubts of infection, inflammation, or disease-induced leukocytosis, thus suggesting an alternative underlying cause for the detected total leukocyte- and neutrophil-level modulation.

HDL-profile

In the past, HDL-C has been depicted as potentially one of the strongest epidemiological surrogates for protection against CVD [222,223]. HDL

functionality is closely connected to the lipid and protein composition, quality, and molecular cargo associated with HDL particles [224]. In regard to HDL particle size, it has been shown that elevated levels of smaller HDL particles are correlated with increased risk of CVD, whereas larger HDL particle size reduces the risk of CVD and associates with metabolically healthy lean phenotype [225,226]. Regarding HDL particle composition, increased levels of apoA1 and phospholipid content of HDL particles have been associated with enhanced reverse cholesterol transport, and thus reduced risk of CVD. Regardless of the arising evidence on HDL composition and functionality studies, it has been suggested that HDL-C and HDL particle number might still be superior compared to HDL particle size in terms of CVD risk prediction [227]. However, human genetic Mendelian randomization and pharmacological studies aimed at increasing HDL-C levels have introduced controversy regarding the causality of HDL-C levels and CVD risk [228,229].

Consequently, in light of the current knowledge, enhanced atheroprotective functionality of HDLs following substantial weight loss, even in normal-weight individuals, was suggested as increased levels of HDL-C, apoAI, HDL particle size, particle number of large HDLs, and HDL phospholipids was detected in the Female Physique Athlete Study (paper I). Subsequently, enhanced atheroprotective functionality of HDLs was also suggested after the RT intervention in the Male Resistance Training Study as increased phospholipid content of large HDLs was detected. In the Female Physique Athlete Study (paper I), observed metabolomic findings were further supported by changes in the regulating factors of lipid metabolism (e.g., LCAT, PLTP, ANGPTL3, ANGPTL4) and mRNA expression levels (e.g., *ABCA1*, *ABCG1, SCARB1*) from transcriptomics. In support of these findings, evidence of similar modulations in HDL profile was suggested after the long-term weight loss in the age- and BMI-matched females from the general population after a 7-year follow-up.

In summary, these results reinforce each other and findings from previous compositional studies on HDLs atheroprotective functionality, and suggest that the manner by which weight loss modulates the composition of HDL profile can be potentially generalized across individuals with different body weight, even below normal levels of fat mass. However, in light of the arising evidence from Mendelian randomization and pharmacological studies, it can be speculated as to whether HDL is causal or actually just a marker for CVD risk.

Non-HDL cholesterol profile

It has been thoroughly covered that negative modulation of cholesterol and lipoprotein metabolism, particularly increased levels of non-HDL cholesterol, promote atherogenic actions, and increase the risk of future CVD [230,231]. Consequently, in the Male Resistance Training Study, the RT intervention induced a reduction in cholesterol content of apoB-containing lipoproteins, apoB, and circulating levels of free cholesterol and remnant cholesterol, thus suggesting attenuated risk of future CVD outcomes. In the past, in accordance with this thesis' findings, RT has been shown to elicit similar beneficial changes in serum non-HDL profile in response to short- and long-term interventions [127,232]. Evidence has implied regarding RT that increased volume, rather than increased intensity has greater positive impact on lipid profile, thus suggesting that volume orientated training regimens should be engaged in if wishing to pursue anti-atherogenic changes in lipid profiles.

Together with exercise training, lean phenotype and weight loss have also been associated with anti-atherogenic attenuation in cholesterol and TG content of VLDLs [233]. Conversely, findings from the Female Physique Athlete Study (paper I) suggested a potentially adverse modulation of non-HDL profile as increased levels of serum-free cholesterol and cholesterol content of VLDLs were evident, together with diminished TG content of VLDLs. Altogether, it can be hypothesised that the VLDL compositional

change towards reduced TG/cholesterol ratio is caused by weight-loss induced RCT and lipolysis, that were suggested by transfer protein activity (e.g., CETP ↑) and mRNA expression levels (e.g., *LPL* ↑). Specifically, CETP is able to promote cholesterol/TG transfer between large buoyant HDLs and VLDLs, whereas LPL participates in the degradation of VLDL TG content [57].

In concordance with these findings, evidence of anti-atherogenic modulations of non-HDL profile regarding lipoprotein diameter (e.g., LDL ↑, VLDL ↓), cholesterol content (e.g., LDL ↓, VLDL↑), and particle numbers (e.g., LDL ↓, VLDL ↓) was suggested after the long-term weight loss in the age- and BMI-matched females from the general population after a 7-year follow-up. When comparing these DILGOM 2007 Study individuals with physique athletes, similar differences in non-HDL profile were observed regardless of matching based on BMI and age. This finding was most strongly explained by differences in body composition, thus contributing to the current body of evidence that detailed adiposity measures seem to be more effective in predicting cardiometabolic status compared to BMI alone [234-237].

Triglyceride profile

In the past, weight loss in overweight individuals has been shown to diminish endogenous hepatic TG production, hepatic inflammation, and TG-enriched VLDL secretion from the liver [238]. As mentioned earlier, TG content of VLDLs, the major location of serum TG levels and subsequently a risk factor for CVD, was reduced along with the size of VLDLs as a result of weight loss in the Female Physique Athlete Study (paper I). These findings were further enforced by the increased expression of *OSBPL10*, a gene responsible for coding the hepatic lipogenesis inhibiting protein, ORP10, which has the potential to reduce TG-rich VLDL production from the liver [239,240].

In contrast with the TG content of VLDLs, increased TG content of HDL and LDL were observed, a potentially adverse modulation observed in

individuals with excess adiposity and metabolic syndrome, that might impair several lipoprotein functions, thus enhancing their atherogenic effect and increasing the risk of future CVD [241]. However, TG-enrichment of HDL and LDL particles was detected concomitantly with increased particle diameter in both lipoprotein subclasses – modulation that is associated with diminished risk of CVD [242,243]. It is also well established that reduced TG content of VLDL (similar to findings in the Female Physique Athlete Study) result in the production of larger, more TG-enriched LDLs [244,245]. Large TG-rich HDLs and LDLs can also function as beneficial substrates for lipid regulating enzymes and receptors (e.g., PLTP, LPL, LPR). Subsequently, following the weight loss upregulation of these lipid metabolism modulators was suggested by increased expression of corresponding genes, thus promoting the view of enhanced TG clearance and advantageous TG shift between lipoproteins and tissues. Altogether, TG enrichment of HDL and LDL lipoproteins was not reflected on the total levels of lipoprotein TGs, thus attenuating any further doubts of adverse effects on future cardiovascular health. In concordance with these findings, similar TG profile modulation by a short-term RT regimen was reported in the Male Resistance Training Study as increased TG content of IDLs and LDLs were detected, together with nominal findings implying diminished TG content of VLDLs and TG enrichment of HDLs.

In summary, weight loss and RT in normal-weight individuals seem to promote TG shift from VLDLs to LDL (and IDL) and HDL lipoproteins. However, additional proteomic and detailed compositional analysis studies should be pursued to examine the lipoprotein particles generated during different physiological states that promote TG clearance and TG shift between lipoproteins (e.g., weight loss, RT). These studies should re-evaluate whether TG enrichment of LDL and HDL lipoproteins has adverse implications only when in presence with elevated overall TGs.

Serum free fatty acid profile

Recently published evidence suggests that cardiometabolically beneficial FFA profiles are characterised by overall reduced levels of FFAs together with increased levels of serum ω-3 PUFAs and CLAs, and reduced levels of MUFAs and trans-FFAs [154,157,158,246,247]. However, to date, evidence on the effects of body composition and exercise training modulation of the serum fatty acid profile is somewhat contradictory and scarce, especially regarding circulating levels of SFA and ω-6 PUFA.

Thus, in light of the current knowledge, it is hard to predict potential cardiometabolic effects caused by the substantial weight-loss induced increase in SFAs and attenuation in degree of FFA unsaturation observed in the Female Physique Athlete Study (paper I). Interestingly, in obese individuals, similar observations have been documented where weight loss has resulted in attenuated levels of unsaturated FFAs together with increased SFAs [154]. Furthermore, although, dietary and circulating SFAs have been generally thought to have detrimental effects on health, it has been reported that unsaturated FFAs correlate more strongly with metabolic markers compared to SFAs [154]. In the future, more information is needed by methods that can separate individual FFA characteristics in more detail (e.g., mass-spectrometer metabolome) as it has been suggested that these disparities in FFA profile findings, especially regarding SFAs, are caused to some extent by differences in fatty-acid length and composition [154]. For example, odd chain and very-long-chain SFAs have been associated with reduced risk of adverse metabolic outcomes, whereas even-chain SFAs have not [154,248].

Moreover, findings from the Male Resistance Training Study implied RT to have a cardiometabolically beneficial effect on circulating FFA profile, as increased levels of CLA were detected. However, it has been reported that serum CLA levels are mostly affected by dietary intake (e.g., milk, meat, supplementation) and biosynthesis by intestinal microbiota [249]. Subsequently, modulation of these effectors is most likely the reason for observed alteration in serum CLA content. As exercise training is known to

modulate gut microbiota [250,251], it can be speculated as to whether the observed increase in circulating levels of CLA could be caused by RT-induced changes in gut microbiota or potentially through changes in dietary intake. Altogether, regardless of the origin of the increased serum CLA content, higher levels of circulating CLA are connected with reduced risk of adverse health outcomes, although the evidence is sparse and should be confirmed by future studies [160].

Serum amino acid profile

In the past, higher levels of aromatic, branched-chain amino acids, and alanine have associated with obesity and molecular pathways linked to increased risk of metabolic disorders in large population-based cohorts [252-254], whereas increased levels of amino acids, glutamine and glycine, have associated with low BMI and healthy metabolic function [126]. Consistent with these findings, cardiometabolically advantageous modulation of serum amino acid profile was suggested following the intense weight-loss period in the Female Physique Athlete Study (paper I) as increased levels of glutamine and glycine, together with declined levels of the aromatic amino acid, histidine, were detected. Moreover, a contradictory elevation in serum alanine levels was also observed following the intense weight-loss period, thus raising doubts of potential disruption of metabolic health. However, it has been shown that during times of intense exercise training and caloric restriction, glucose-alanine cycle promotes endogenous alanine production [255]. A mechanism most probably explaining the elevated levels of alanine in consequence to energy deprivation and intense exercise training in the Female Physique Athlete Study (paper I).

Similar to the intense weight-loss period in the Female Physique Athlete Study (paper I), RT intervention in the Male Resistance Training Study also resulted in a presumably cardiometabolically advantageous elevation in levels of serum glutamine. Conversely, a potentially adverse increase in serum aromatic amino acids, phenylalanine and tyrosine, was

observed as a consequence of the RT intervention. Doubts in relation to adverse health effects were alleviated, however, by previous reports that have documented long-term exercise training to induce similar modulations in serum aromatic amino acid profile [150,256]. Furthermore, evidence is growing in relation to the efficacy of RT in promoting metabolic health through glycaemia control and treatment of T2Ds, even during ab libitum diet, thus diminishing further doubts of RT-induced metabolic dysfunction [257,258].

Our observation of altered amino acid concentrations in both studies were probably mediated through altered amino-acid and protein metabolism due to RT and altered energy availability and subsequent change in adiposity. It is also plausible that changes in serum–amino-acid profiles were affected by the amount of dietary protein, although no significant changes were observed in self-reported dietary values that might however possess some uncertainty. Altogether, based on findings from this thesis, it can be argued that the use of circulating levels of amino acids as disease predictors cannot be ultimately generalized to all populations, as they are highly dependent on age, energy availability, dietary protein content, and exercise/activity level.

6.2 Systems biology of weight (re)gain in normal-weight young individuals

Weight gain is a significant risk factor negatively affecting overall health on multiple levels of physiology, but it has remained unclear whether weight loss followed by weight regain (i.e., weight cycling) in normal-weight individuals has any deleterious health implications. The prevalence of weight loss attempts and trendy crash diets in modern society is increasing, even among individuals within a normal-weight range [259]. As such, insights into the possible health effects of weight loss and subsequent weight regain (e.g., weight cycling, transient weight loss) among a normal-weight population are greatly needed.

From an evolutionary stand point, weight cycling must have been evident throughout the majority of human history, since people have regularly undergone periods of hunger when food supply has been limited (e.g., winter, drought), which would imply doubt as to whether substantial detrimental effects can be expected to emerge as a results of weight cycling. To date, however, some studies have indeed observed that weight cycling appears to induce adverse health effects and increase the risk of morbidity, although evidence is sparse [75,260]. There is also contradictory emerging evidence that caloric restriction appears to have numerous beneficial effects on both health and lifespan [261]. Thus, it stands to reason whether fasting episodes or episodes of caloric deficit may be even beneficial for humans by resetting metabolomic processes or perhaps by recycling proteins. To date, however, the molecular mechanisms mediating the beneficial effects of caloric restriction are relatively unknown, although mitochondria that act as central bioenergetic organelles in the cells, are thought to play an important role in caloric-restriction induced metabolic adaptations [262]. In the future, it should be elucidated in more detail whether these potentially beneficial physiological changes induced by caloric restriction/weight loss persist or are diminished in response to increased energy intake and weight regain.

In the Female Physique Athlete Study, even modest long-term positive effects of weight loss were suggested on the HDL profile. However, it can only be speculated whether these beneficial effects in HDL profile were observed due to the potentially insufficient length of the weight-regain period and/or the amount of weight regained by the end of the study, or whether these changes will persist for longer periods. Overall, this thesis demonstrated that weight regain after substantial weight loss normalizes the majority of the observed changes in measured systems biology markers even in normal-weight individuals, thus alleviating doubts of weight cycling related long-term positive/adverse health effects within normal-weight population [75]. The aforementioned findings on cardiometabolic signatures

following weight (re)gain among normal-weight individuals were corroborated, as similar changes in metabolomic profile (e.g., HDL and lipid profile) were detected after long-term weight gain in the age- and BMI-matched females from the general population after a 7-year follow-up.

Although a majority of the cardiometabolic factors, including HDL profile, lipid levels, and systemic inflammation, returned to baseline levels following weight regain in the Female Physique Athlete Study (paper I), a notable upregulation in genes associated with adverse cardiovascular outcomes and blood-derived signals was observed after the weight-cycling period (e.g., platelet activation, hypertrophic and dilated cardiomyopathy). These pathways were unaffected by the preceding weight loss, only by the weight regain, despite body weight levels not being restored above baseline levels. Previous studies investigating gene expression profiles in obesity and during periods of weight gain have reported similar expression patterns on the same cardiac failure associated pathways [14,263], thus supporting the findings from the Female Physique Athlete Study (paper I). Moreover, these results seem to imply that weight loss and weight (re)gain may have selective independent effects on the induction of different cardiovascular gene pathways. It can also be speculated whether the limited selection of omic domains investigated in this thesis have been unable to capture some other adverse physiological changes associated with substantial weight loss and subsequent regain. Thus, in the future, other tissues (e.g., fat, liver, muscle, stool) and/or omic domains (e.g., microRNA, DNA methylation, microbiome) should be examined in a similar study setting to unravel any unforeseen physiological changes of yo-yo dieting.

Overall, the results of this thesis suggest that weight-cycling–associated weight (re)gain from low levels of body weight, regardless of i) preceding weight loss, ii) low starting weight, and iii) magnitude of weight (re)gain, might have a negative impact on CVD risk and heart failure related gene pathways. More studies are needed, however, to validate these adverse findings and to determine whether subsequent weight cycling has any

tangible implication in terms of overall health. Despite the deleterious findings, it should be kept in mind that when compared to age- and BMI-matched general population individuals, physique athlete cardiometabolic signatures were preceding weight loss already at more favourable levels regarding all aspects of lipoprotein and TG profile and systemic inflammation. To conclude, the majority of the observed weight loss induced systems biology changes were indeed reverted back to baseline in the Female Physique Athlete Study, thus diminishing further doubts of major long-term health effects caused by physique competing associated weight cycling.

6.3 Phenotype and lifestyle factors as independent determinants of systems biology

During the ongoing omics era, a growing number of studies have elucidated aspects of how different phenotype and lifestyle characteristics (e.g., age, gender, diet, exercise training, body composition) modulate systems biology. To date, however, the independent effects of body composition, exercise training, and diet on systems biology are relatively controversial due to the high inter-correlation between these factors. Thus, distinguishing any independent effects on physiology is rather cumbersome.

Body composition

It has been suggested that rather than changes in overall body weight and adiposity, specific reductions in visceral fat mass and abdominal adiposity might explain most of the improvements seen in lipid profile and low-grade inflammation following periods of exercise training and weight loss [264-267]. The present findings from this thesis reinforced this view as advantageous cardiometabolic changes in lipid profiles and inflammation-related biomarkers were mostly strongly explained by changes in visceral fat mass rather than changes in other fat mass compartments, exercise level, or energy intake in the Female Physique Athlete Study (paper I). As these

weight-loss induced anti-atherogenic changes in serum metabolome occurred even in athletes with normal levels of fat mass, it seems that the observed correlations from the overweight range also hold in lean individuals with low levels of fat mass. In accordance with these observations, even preceding weight loss, physique athletes who maintain a body composition characterised by lower levels of fat mass outside the competition season demonstrated more favourable levels of several health-related biomarkers, including lipid levels, HDL profile, and inflammation-related biomarkers compared with age- and BMI-matched females from the general population, thus demonstrating the benefits of maintaining lower levels of body fat even within the range of normal BMI.

In summary, alterations in body composition, namely fat mass quantity and distribution, most accurately explained the observed modulation in cardiometabolic profiles assessed in the Female Physique Athlete Study (paper I). Thus, it can be speculated that exercise training and energy deficit are the main effectors of fat mass loss, facilitating the observed changes in cardiometabolic profile. However, energy intake and exercise training volume/intensity were based on self-reported values that could potentially introduce uncertainty and bias into these findings. Generally, individuals tend to overestimate physical activity levels and underestimate energy intake levels that could have contributed to the rather weak association detected between these effectors and cardiometabolic signatures. Although the results of this thesis reinforce the superior role of especially fat mass in the prediction of biomarker modulation, they also demonstrate the challenges of using BMI as a predictor of cardiometabolic profile compared to fat mass levels and more accurate measures of adiposity.

Resistance training

Exercise training has been shown to beneficially alter serum HDL subpopulation profiles in previously untrained people independent of

weight change [150,268]. Subsequently, independent of weight change, similar anti-atherogenic effects on cardiometabolic profiles were observed following the 16-week RT intervention in the Male Resistance Training Study. However, assumptions of RT-derived independent effects on cardiometabolic profiles were diminished as a significant increase in lean mass and decreased level of overall and visceral fat mass were observed regardless of the unaltered body weight. The detected augmentation in levels of lean mass was most significantly associated with increased levels of HDL subpopulations, especially among high responders that adhered to the more volume orientated training group. In the past, both fat mass loss and volume orientated RT has also been shown to associate with similar beneficial effects of HDL profile [127]. Thus, despite the significant association between lean mass and HDL subpopulation levels in the Male Resistance Training Study, it is plausible and even likely that concomitant reduction in fat mass and increased volume of RT to some extent confound the association.

In the future, instead of weight and BMI, more accurate measures of body composition should be used when trying to determine exercise-training–dependent effects on cardiometabolic profile and risk markers, such as visceral fat mass, overall fat mass, and lean mass. Subsequently, as engagement in exercise training has a tendency to increase lean mass and promote loss of fat mass, it can be argued that the use of body weight and BMI as covariates is not sufficient for capturing exercise training dependent effects on physiology. Furthermore, it has been documented that when pursuing weight loss, dietary energy restriction has a superior effect on total body weight lost compared to exercise training alone [269]. Exercise training has a greater potential, however, in diminishing visceral fat mass content even in the absence of weight loss. Nevertheless, it should be noted that exercise training and energy intake are indeed important factors in mediating body composition changes, thus their effects cannot be completely excluded when examining associated physiological changes.

Baseline phenotype

Previous studies have shown that the individuals most favourably affected by exercise regimens are those with most adverse baseline phenotype characteristics and biomarker levels (e.g., low HDL-cholesterol levels, increased abdominal adiposity, and elevated serum TG levels) [270]. Responsiveness to exercise training has also been reported to be dependent to some extent on individual endogenous characteristics such as age, gender, and genetics [271,272]. Subsequently, the most distinctive cardiometabolic profile modulation by RT in those with the largest changes in lean mass (i.e., high-responders) was probably mostly explained by the aforementioned observations as the high-responders in the Male Resistance Training Study had lower levels of lean mass, higher levels of fat mass, and lower HDL-cholesterol levels compared with the low-responders at baseline. This shows that improvements in HDL-cholesterol levels and body composition are most evident in people with the most unfavourable baseline levels. Low-responders had also greater variance in the metabolic profile, which could have to some extent potentially contributed to the differences found when compared to the high-responder group. Overall, this thesis' findings support the recommendation that suitable exercise regimens should be targeted especially to people with the poorest health parameters concerning both body composition and metabolic profile. Recently, it has also been questioned whether responders/non-responders to exercise training do actually exist or whether inter-individual variation is caused by differences in individual responsiveness to specific modalities (intensity, volume, or duration) [273].

6.4 Limitations

Studies presented in this thesis had some limitations discussed below. Regardless of the fact that the sample sizes were relatively modest, a longitudinal design was used and thus in theory rather strong statistical

power was achieved as demonstrated by previous omics studies [150]. In addition, much larger sample sizes were used when compared to previous studies where physique athletes or RT have been investigated. Similar studies with larger sample sizes are, however, warranted in order to validate the efficacy of the sample sizes and power to capture the biological variance in the measured variables. It is also recognized that drop-outs in the Female Physique Athlete Study (n = 10) and the Male Resistance Training Study (n = 9) could also potentially introduce attrition bias into the results of this thesis. To minimize potential bias caused by attrition and differences in study group sizes, statistical analyses in this thesis study was primarily focused on exploring within-group analyses in both the Female Physique Athlete Study and the Male Resistance Training Study. Because of availability of the repeated measurements within each individual, each study participant acts as his/her own control, and hence such within-group analyses avoid potential problems due to insufficient matching of case and control groups. Within group analyses were also pursued in the Female Physique Athlete Study due to some of the observed baseline differences (e.g., physical activity) between the diet and control group. The phenotypes in terms of age and body composition were, however, very similar to one another, which is the reason for why the pooling of both groups was justified for the comparison analysis between the physique athletes and age- and BMI-matched general-population individuals. Lastly, it is recognized that having investigated both sexes in two different study designs requires that some caution be used when interpreting and comparing the results of both studies of this thesis.

The lack of dietary standardization can also be recognized as a limitation of this thesis study, as dietary intake is known to significantly affect the metabolomic profile, especially serum lipid and amino acid levels. In studies similar to this one that examined exercise training and dietary responses, as well as associated changes in body composition, it is hard to determine the independent effects of body composition changes, diet, or

exercise training on the quantified systems biology markers due to the high inter-correlation between the factors. Moreover, accuracy of self-reported dietary information is not usually entirely reliable, thus dietary data collected and used as covariates in this thesis could potentially introduce bias to the analyses. However, in the Female Physique Athlete Study, physique athletes follow strict dietary regimens/program and track dietary intake via a food scale, thus dietary information can be considered relatively objective. Conversely, self-reported *ad libitum* dietary information in the Male Resistance Training Study is more likely to include more bias, which is the reason dietary information was not utilized as a covariate in the primary analyses. In addition, coverage of the food item database in the used dietary analysis software (AivoDiet, Nutriflow) can also result in inaccurate calculations and bias the observed dietary composition of study individuals. It is also recognized that in addition to dietary composition, other lifestyle factors (e.g., sleep, stress) that were not accounted for in this thesis might contribute to some extent to the changes observed in the various quantified omics dimensions.

In the Female Physique Athlete Study, no available reported subjective data was available on incidence of infections or infection symptoms, from the participants to further evaluate immunosuppression and effects of the physiological changes on health. In addition, more accurate flow cytometry analysis of WBCs is warranted in future studies to validate the changes indicated by the transcriptomic data in white blood cell populations. The lack of measured resting energy expenditure is also recognized as a possible limitation as differences at baseline metabolic rate may contribute to the immunometabolism responses of the diet group participants when subjected to prolonged intense exercise training and low energy availability regimens.

Although blood as a choice of tissue has several benefits as discussed earlier, it has to be acknowledged that blood-derived systems biology markers do not necessarily reflect changes occurring in other tissues that

may be highly informative when assessing the effects of diet and exercise, such as muscle, fat, liver, stool, and urine. However, in a study setting with repeated measures, the invasiveness of procedures to obtain muscle, fat, or liver biopsies might generate ethical issues and/or make it harder to recruit individuals.

Although a wide array of blood-derived systems biology factors was quantified in this thesis to determine changes occurring in cardiometabolic and immune-system signatures, it is highly likely that some undetected physiological changes could have been captured by examining other blood-derived omic dimensions more extensively, such as microRNAs, DNA methylation, and proteomics. In the future, these omic dimensions could be explored in more detail in a similar study setting to determine any physiological changes that were not captured by the omics categories used in this thesis. Moreover, on the methodology of this thesis, it should be noted that mRNA expression levels derived from leukocytes may not necessarily reflect actual levels of biologically-active proteins in tissues that are important for their physiological function. In addition, in the end, it is highlighted that despite benefits of having used the targeted H-NMR-metabolomics platform in the Female Physique Athlete Study, the Male Resistance Training Study, and DILGOM 2007 and 2014 Studies, it has limited sensitivity and coverage when compared to metabolomics approaches utilizing mass spectroscopy. Also, it should be noted that a number of the measured lipoprotein species are difficult to interpret as they are comprised of heterogeneous particle distributions.

Lastly, it should be kept in mind that the studies of this thesis investigate correlations/association between changes in body composition, exercise training, and energy availability together with changes in blood-derived biomarkers, and results should be interpreted with caution since actual causal relationships between biomarkers and clinically relevant phenotype outcomes are not always certain.

7 MAIN FINDINGS AND CONCLUSIONS

The main objectives of this thesis were to evaluate the effects of body composition, exercise training, and energy availability on the systems biology of healthy normal-weight individuals. Prior to this thesis, it has been extensively covered that weight loss, exercise training, and reduced energy intake effectively rehabilitate obesity related disrupted metabolic homeostasis and improve future prognosis. However, it has not been studied whether these beneficial effects of weight loss, exercise training, and reduced energy intake can be generalized to the physiology of normal-weight individuals wishing to further promote well-being and longevity. The studies of this thesis address these gaps in knowledge and demonstrate:

1) Significant weight loss leading to substantial visceral fat mass reduction through high levels of exercise and energy restriction can further improve cardiometabolic profile through modifying serum lipid levels, HDL profile, and inflammation-related biomarkers, even in normal-weight females.
2) Compared to BMI or weight alone, adiposity level and fat mass distribution predict more efficiently cardiometabolic profile, in normal-weight females.
3) Weight gain, even from low levels of fat mass, induced atherogenic and heart-failure–related gene pathways, thus demonstrating that regardless of starting weight, the detrimental effects of weight gain can be observed in the cardiometabolic profile.
4) Prolonged periods of low-energy availability and a high amount of exercise leading to weight loss have a significant effect on multiple levels of the immune system, specifically, dysregulated haematopoiesis, suppressed immune cell proliferation/maturation, and loss of function were suggested by the altered immune-system signatures.

5) Weight regain from low levels of fat mass reverts the majority of the physiological changes caused by preceding substantial weight loss and better CVD health markers in physique/fitness competitors when compared to matched controls attenuate doubts of major long-term negative health effects caused by physique competing.
6) Sixteen weeks of RT leading to increased levels of lean mass and reduced overall adiposity lead to anti-atherogenic modulation of the serum metabolome: specifically, a decreased pool of non-HDL cholesterol and subsequent modified apolipoproteins, even in healthy young men.
7) Individuals with the poorest baseline body composition and biomarker profile are most responsive and benefit the most from RT regimens in terms of beneficial cardiometabolic health effects.

Overall, this thesis presents that weight loss, exercise training, and reduced energy intake lead to beneficial modulation of cardiometabolic profile, even in healthy normal-weight individuals. However, more studies are needed to ascertain whether further beneficial alteration of cardiometabolic profile has any tangible implications in terms of cardiovascular health in normal-weight population. More studies are also warranted to ascertain and examine the effects of repeated weight loss bouts and subsequent weight regain on cardiometabolic profile and health, and especially to immune-system signatures and function to elucidate possible susceptibility to autoimmune dysregulation. Findings of this thesis together with future studies can help to guide future exercise and dietary recommendations for normal-weight individuals (e.g., athletes, general population) wishing to aim for more aesthetic appearance, well-being, and longevity.

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